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STUDIES OF THE ECOLOGICAL IMPACT OF REPETITIVE AERIAL APPLICATIONS OF HERBICIDES ON THE ECOSYSTEM OF TEST AREA C-52A, EGLIN AFB, FLORIDA

AIR FORCE ARMAMENT LABORATORY, EGLIN AIR FORCE BASE, FLORIDA

AUGUST 1975

AFATL-TR-75-142

STUDIES OF THE ECOLOGICAL IMPACT OF REPETITIVE AERIAL APPLICATIONS OF HERBICIDES ON THE ECOSYSTEM OF TEST AREA C-52A, EGLIN AFB, FLORIDA

ENVIRONICS AND HUMAN FACTORS OFFICE
AIR FORCE ARMAMENT LABORATORY
AND DEPARTMENT OF CHEMISTRY AND BIOLOGICAL SCIENCES
UNITED STATES AIR FORCE ACADEMY

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EGLIN AIR FORCE BASE, FLORIDA

prior to the arrival of the sonic boom's audible report and overpressure signature measurement, were identical to the first obtained. Further study disclosed that this response of songbirds coincides with the arrival of the seismic signal propagated through the ground and preceding the sonic boom shock wave by 4 to 8 seconds. (Modified author abstract)

AD-780 117/8GA PC$3.75/MF$1.45
Stanford Research Inst Menlo Park Calif
DEVELOPMENT OF MOSQUITO REPELLENTS HAVING INCREASED PROTECTION TIME IN MAN.
Annual rept. 1 Jul 73-15 Mar 74,
W. A. Skinner, Howard L. Johnson, and H. C. Tong. 15 Mar 74, 66p
Contract DADA17-70-C-0112

Descriptors: *Culicidae, *Insect repellents, Ethers, Behavior, Humans, Protection, Glycols, Thiazoles, Quinolines, Sulfonamides, Toxicology, Humans.
Identifiers: *Mosquitoes. Aedes aegypti, Quinoxalines, Thiazolidinones, Polyethers.

Synthetic topical repellents were prepared, following previously reported leads that resulted from intrinsic repellency studies and from Stanford Research Institute (SRI) computed-coded USDA compounds. The most promising structural area for topical repellency continues to be glycol ether derivatives, although the carbamide, 2,4-thiazolidinedione, and quinoxaline areas are also of interest. Efforts were directed toward polymer formulations of SRI glycol ethers to improve their wash-off characteristics and cosmetic acceptability. These studies range from the typical film-formers to carbowax stick-type formulations. Large samples of two 2,4-thiazolidinediones were synthesized for toxicologic evaluation. (Modified author abstract)

AD-780 466/9GA PC$3.00/MF$1.45
Arizona Univ Tucson Dept of Entomology
BIONOMICS OF MOSQUITOES IN SEMI-ARID URBAN AREAS.
Final rept. 1 Jul 73-30 Jun 74,
John L. McDonald. 15 Jun 74, 14p
Contract N00014-74-C-0017

Descriptors: *Culicidae, *Arizona, *Insect control, Bites and stings, Disease vectors, Encephalitis, Deserts, Arid land, Urban areas.
Identifiers: Psorophora signipennis, Psorophora confinnis, Aedes vexans.

Incidence of biting mosquitoes in the semi-arid urban areas, as represented by Tucson, Arizona, is a function of temperature and rainfall. Eight species of mosquitoes were collected during the year but biting mosquito problems were only associated with three species: Psorophora signipennis, P. confinnis and Aedes vexans. Significant mosquito control was realized using mosquito fish (Gambusia affinis) alone and/or in combination with Flit MLO and Dursban. (Author)

AD-780 517/9GA PC$4.75/MF$1.45
Air Force Armament Lab Eglin AFB Fla
ECOLOGICAL STUDIES ON A HERBICIDE-EQUIPMENT TEST AREA (TA C-52A), ELGIN AFB RESERVATION, FLORIDA.
Final rept. Jan 67-Nov 73,
Alvin L. Young. Jan 74, 141p Rept no. AFATL-TR-74-12

Descriptors: *Herbicides, *Ecology, Drainage, Soils, Degradation, Plants(Botany), Animals, Microorganisms, Test facilities.
Identifiers: Pesticide residues.

The report attempts to answer the major questions concerned with the ecological consequences of applying massive quantities of herbicides (346,117 pounds), via repetitive applications, over a period of eight years, 1962 - 1970, to an area of approximately one square mile. Moreover, the report documents the persistence, degradation, and/or disappearance of the herbicides from the Test Area's soils and drainage waters and their subsequent effects (direct or indirect) upon the vegetative, faunal, and microbial communities. The active ingredients of the four military herbicides (Orange, Purple, White, and Blue) sprayed on Test Area C-52A were 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-amino-3,5,6-trichloropicolinic acid (picloram), and dimethylarsinic acid (cacodylic acid). (Modified author abstract)

AECL-3956 PC$6.00/MF$1.45
Atomic Energy of Canada LTD., Pinawa, Manitoba. Whiteshell Nuclear Research Establishment.
HABITATS OF SMALL MAMMALS AT WHITESHELL NUCLEAR RESEARCH ESTABLISHMENT.
S. L. Iverson, and B. N. Turner. Dec 73, 58p

Descriptors: (*Canada, *Ecology), (*Mammals, *Distribution), Conifers, Environment, Forests, Herbs, Reactor sites, Sampling, Shrubs, Trees, Wr-1 reactor.

For abstract, see NSA 29 12, number 29395.

CEA-CONF-2517 PC$3.00/MF$1.45
Institut National de la Sante et de la Recherche Medicale (Inserm), 06 - Nice (France). Centre d'Etudes et de Recherches Medicales de Biologie et d'Oceanographie Medicale (Cerbom).
EXPERIMENTAL INVESTIGATIONS, AT LABORATORY, ON THE TRANSFER OF MERCURY IN MARINE TROPHIC CHAINS.
F. Laumond, M. Neuburger, B. Donnier, A. Fourcy, and R. Bittel 1973, 6p CONF-730995-1

Descriptors: Aquatic ecosystems, Diffusion, Fishes, Food chains, Mercury, Molluscs, Plankton, Seas, Seawater.

For abstract, see NSA 29 12, number 31629.

COM-74-11057/8GA PC$20.00/MF$20.00
National Technical Information Service, Springfield, Va.
SEWAGE EFFECTS IN MARINE AND ESTUARINE ENVIRONMENTS. A BIBLIOGRAPHY WITH ABSTRACTS.
Rept. for 1974-May 74,
Edward J. Lehmann. Jun 74, 117p* NTIS-WIN-74-047

Descriptors: *Bibliographies, *Sewage, *Water pollution, *Sludge, *Aquatic biology, Oceans, Ecology, Aquatic biology, Sewage disposal, Sludge disposal, Estuaries, Water quality, Benthos.
Identifiers: *Water pollution effects(Animals), *Water pollution effects(Plants), *Ocean waste disposal.

The bibliography contains 112 selected abstracts of research reports retrieved using the NTIS on-line search system - NTISearch. The topics cover the effects that sewage effluents and sludge have upon marine and estuarine environments especially on their ecology. Included are reports dealing with the effects on marine plants and animals, problems due to ocean dumping, dispersion studies, water chemistry, and other related topics.

COM-74-11091/7GA Reprint
Wisconsin Univ., Madison. Sea Grant Program.
FIELD STUDIES ON PHOTOSYNTHESIS OF 'CLADOPHORA GLOMERATA' (CHLOROPHYTA) IN GREEN BAY, LAKE MICHIGAN,
Michael S. Adams, and Walter Stone. 1973, 13p WIS-SG-74-342 NOAA-74051603
Pub. in Ecology, v54 n4 p853-862 Summer 73.

Descriptors: *Chlorophyta, *Photosynthesis, *Algae, *Green Bay, Lake Michigan, Aquatic plants, Nutrients, Wisconsin, Ecology, Nitrogen, Calcium, Strontium, Sodium, Zinc, Sunlight, Temperature.
Identifiers: Sea Grant program, *Cladophora glomerata, *Eutrophication.

Net photosynthesis of Cladophora glomerata was measured at three sites in lower Green Bay, Lake Michigan, from late spring through summer, 1971. Lower levels of productivity occurred early in the season at two of the sites, when water temperatures were lowest. At the third site water temperature and productivity varied the least. Contrary to other reports, it was found that Cladophora made relatively efficient use of low illumination. Under statistically similar temperature and irradiance levels, productivity was higher with increasing proximity to the mouth of the Fox River. Nitrogen, calcium, strontium, sodium, and zinc also were highest in concentration in plants receiving the greatest amount of effluents from the Fox River in comparison with the site receiving the least. Site differences in productivity appear to be related to nutrient levels, whereas seasonal differences in productivity are probably most closely related to seasonal temperature differences.

ORNL-NSF-EATC-6 PC$24.00/MF$1.45
Oak Ridge National Lab., Tenn. (Usa).
ECOLOGY AND ANALYSIS OF TRACE CONTAMINANTS. PROGRESS REPORT, JANUARY 1973--SEPTEMBER 1973.
W. Fulkerson, W. D. Shults, and R. I. Van Hook. Jan 74, 438p
Contract W-7405-eng-26

Descriptors: (*Ecology, *Research programs), (*Environment, *Pollution), Aquatic ecosystems, Electrochemistry, Food chains, Gas chromatography, Hydrology, Information retrieval, Information systems, Liquid wastes, Multi-element analysis, Ornl, Solvent extraction, Terrestrial ecosystems, Trace amounts, X-ray fluorescence analysis.

For abstract, see NSA 29 12, number 31535.

PB-232 988/6GA PC-GPO/MF$1.45-NTIS
Oregon State Univ., Corvallis. Dept. of Microbiology.
EFFECTS OF TEMPERATURE ON DISEASES OF SALMONID FISHES.
Ecological research series,
J. L. Fryer, and K. S. Pilcher. Jan 74, 119p* EPA-660.3-73-020
Paper copy available from GPO $1.55 as EP1.23:660/3-73-020.

Descriptors: *Infectious diseases, *Salmon, *Trout, Fishes, Pathology, Mortality, Experimental data, Temperature, Animal diseases, Microbiology, Effluents.
Identifiers: *Thermal pollution, Water pollution effects(Animals), Chondrococcus columnaris, Aeromonas salmonicida, Aeromonas liquefaciens, Ceratomyxa shasta.

The effect of water temperature on infections of salmonid fish was investigated. Chondrococcus columnaris infection was studied in rainbow trout, coho and spring chinook salmon, Aeromonas salmonicida infection in coho and spring chinook salmon; and Aeromonas liquefaciens infection in steelhead trout. In all cases mortality rates were high at 64 to 69F; moderate at 54 to 59F; and low or zero at 39 to 49F. Progress of the infections was accelerated at higher temperatures, and progressively retarded at decreasing temperature levels. In infection of coho with Ceratomyxa shasta, mortality was high at 69F, low at 49 to 54F, and zero at 39 to 44F. This infection in rainbow trout resulted in high mortality at all temperatures except 39F. In both cases the course of the disease was most rapid at higher temperatures, and became progressively slower as the temperature decreased. For infection of kokanee salmon fingerlings with

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Aquatic Studies	Ecological Investigations	2,4-dichlorophenoxy-acetic acid
Blue	Fish	
Defoliant Testing	Herbicide	Ecological Succession

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
Field investigations were conducted on rodents, insects, aquatic organisms, and plant species associated with a unique 1-square-mile military test site (Test Area C-52A, Eglin Air Force Base, Florida) that was sprayed with 160,948 pounds 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and 169,292 pounds 2,4-dichloro-phenoxy-acetic acid (2,4-D). Although neither 2,4-D nor 2,4,5-T residues could be detected in the soils in 1973 or 1974, significant levels (10-710 parts per trillion - ppt) of the contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) were found within the top 6 inches of test site soils although in some instances

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Item 19 (Concluded):

Histology	Necropsy	TCDD
Insect Survey	Orange	Teratogenic
Mammals	Purple	Test Area C-52A,
Military Defoliation Program	Reptiles	Eglin Air Force
Vegetative Coverage Survey	White	Base, Reservation
2,3,7,8-tetrachlorodibenzo-p-dioxin		

Item 20 (Concluded):

10 years had elapsed since the last aerial application of 2,4,5-T.

An in-depth study of the field effects of the herbicide and TCDD was conducted on populations of beach mice, Peromyscus polionotus, and hispid cotton rats, Sigmodon hispidus. Liver tissue from rodents inhabiting the test site contained 210-1,300 ppt TCDD. However, no gross or histological evidence of teratogenesis or toxicity was found in 122 adults and 87 fetuses. An analysis of variance of liver and spleen weights for the beach mouse indicated significant differences between control and TCDD-exposed animals. Analysis of plant seeds revealed no detectable levels of TCDD (minimum detection limit of 1 ppt TCDD). TCDD accumulation in liver tissue was thought to be associated with pelt contamination from burrowing and subsequent ingestion of soil particles via grooming.

Establishment and succession of plant species on areas denuded by repetitive applications of the herbicides were documented. Large seeded grasses (e.g., Panicum lanuginosum and Panicum virgatum) were the first species established on these sites. Annual herbs (e.g., Diodia teres and Hypericum gentianoides) rapidly invaded the low, moist areas between the grass plants. Seasonal trends in the weather components of wind, temperature, and precipitation were more influential in the reestablishment of vegetation than were herbicide residues.

Comparison of the final results in a 1971 and 1973 sweep net survey of the test area indicated that a threefold increase in insects had occurred during the 2-year period. However, there was no change in the community diversity with time. Increase in number of species was correlated with increase in vegetation.

Species diversities and food chain studies were conducted in two aquatic ecosystems draining the test area. Erosion of soil occurred into a pond on the test area and into a stream immediately adjacent to the area. TCDD levels of 10 to 35 ppt were found in silt of the aquatic systems but only at the point where eroded soil entered the water. Species diversity studies of the stream were conducted in 1969, 1970, 1973, and 1974. Insect larvae, snails, diving beetles, crayfish, tadpoles, and major fish species (by body parts) from both aquatic systems were analyzed for TCDD. Species diversity studies indicated no significant change in the composition of ichthyofauna between these dates or a control stream. Concentrations of TCDD (12 ppt) were found in only two species of fish from the stream, Notropis hypselopterus (sailfin shiner) and Gambusia affinis (mosquito fish). Samples of skin, muscle, gonads, and gut were obtained from Lepomis punctatus (spotted sunfish) from the test grid pond. Levels of TCDD in those body parts were 4,4,18, and 85 ppt, respectively. Gross pathological observations of the sunfish revealed no significant lesions or abnormalities.

SUMMARY

In 1962, the Armament Development and Test Center, Eglin Air Force Base, Florida, was tasked with the responsibility for designing, developing, and testing aerial dissemination systems in support of military defoliation operations in Southeast Asia. It was necessary for this equipment to be tested under controlled conditions that were as near to being realistic as possible. For this purpose an elaborate testing installation, designed to measure deposition parameters, was established on the Eglin Reservation with the place of direct aerial application restricted to a highly instrumented area within Test Area C-52A (TA C-52A) in the southeastern part of the Reservation.

A total of 346,117 pounds of herbicides were applied through repetitive aerial applications, over a period of 8 years (1962-1970), to an area of approximately 1 square mile. The active ingredients of the four military herbicides (Orange, Purple, White, and Blue) sprayed on TA C-52A were 2,4-dichlorophenoxyacetic acid (2,4-D), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), 4-amino-3,5,6-trichloropicolinic acid (picloram), and dimethylarsinic acid (cacodylic acid). It was apparent from analysis of remaining Orange herbicide samples and test grid soils that the 2,4,5-T herbicide contained significant levels of the highly teratogenic (fetus deforming) contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). In particular, one 92-acre test grid (Grid 1) used from 1962 through 1964 received 87,186 pounds of 2,4,5-T In 1974, analysis of 6-inch soil cores for TCDD, taken at six locations in this area, indicated wide fluctuations in TCDD concentrations. The results for the uniformily mixed top 6-inch soil cores were 10, 25, 70, 110 and 710 parts per trillion (ppt) TCDD. Further analysis of a duplicate core, obtained from the site having 110 ppt TCDD concentration, indicated that TCDD was stratified within the top 6 inches of soil. The analysis for depths of 0 to 1, 1 to 2, 2 to 4, and 4 to 6 inches resulted in detectable levels of 150, 160, 700, and 44 ppt TCDD, respectively. Thus significant levels of TCDD residue were found 10 years after the last herbicide mission on this grid.

This report documents the quantitative changes that have occurred in the vegetative and insect communities with ecological succession. In addition, detailed chemical and histopathological studies of segments of the rodent and reptile populations from the test site containing significant levels of TCDD in soil (Grid 1) are reported. Species diversity and food chain studies of the aquatic ecosystems associated with TA C-52A are evaluated.

Because of the massive quantities of herbicides applied to TA C-52A, a unique opportunity existed to evaluate changes in vegetative patterns and/or succession. Field data suggested that climatic factors rather than herbicide residue influenced vegetative establishment especially on the southern portion of the test area, e.g., on or near Grid 1. When wind and temperature data, collected from 1963 to 1969, were combined with data on seed dispersal and regeneration potential, it became apparent that unfavorable conditions on the southern portion of the test area existed at the time when seed dispersal and establishment occurred. Nevertheless, when establishment did take place, a

definite sequence in plant succession could be documented. The large seed grasses Panicum virgatum, switchgrass, and Panicum lanuginosum, woolly panicum, were the first perennial species to assume dominance. The annual herbs Diodia teres, rough buttonweed, and Hypericum gentianoides, poverty weed, rapidly spread in the loose soil around and between the dominant grasses. With time, other grasses occupied significant vegetative cover, especially Andropogon virginicus, broomsedge, and Eragrostic refracta, coastal lovegrass. The square foot transect method of analysis indicated that areas having 5 to 20 percent vegetative cover generally contained 14 species (8 grasses, 6 herbs) while an area of 80 to 100 percent vegetative cover generally contained 28 species (8 grasses, 20 herbs). Similarity between seeds most frequently found in rodent burrows and those from species shown to be early dominants in the plant community suggested that rodents may act as biological dispersal agents into areas of low vegetative cover.

Extensive animal studies were conducted during the summer of 1974. Several methods and procedures were used for collecting data on six interrelated parameters. These included extensive histopathologic examination of beach mouse (Peromyscus polionotus) tissues, a study of beach mouse burrow construction and diet, TCDD analysis of mouse liver tissue and pelts, an examination of the soil profile for potential zoning of TCDD, a laboratory experiment to demonstrate one possible route of TCDD uptake by the beach mouse, and an analysis of reptile tissue for TCDD contamination.

A total of 106 adult and 67 fetuses of beach mice were examined grossly and histologically for congenital and teratogenic defects. In general, microscopic examination of all tissues showed only minor and insignificant lesions of the type normally observed when a large group of animals are examined. The only exceptions being two mice with necrotizing hepatitis and one mouse with renal ectasia of one kidney. The hepatitis was considered to be viral induced and the renal ectasia was interpreted as being of little functional significance and probably a result of a unilateral congenital anomaly not related to any toxicity. An analysis of variance of liver and spleen weights indicated significant differences between control and TCDD-exposed animals. However, these differences were not explained by any histologic differences.

Samples of liver and pelts from mice captured in areas in which significant soil levels of TCDD were found, exhibited accumulations of TCDD in the liver from 540 to 1300 ppt, while pelt contamination was found to be 130 to 140 ppt.

A study of the beach mouse burrows and diet, combined with the findings that TCDD was confined to the top 6 inches of soil, suggested that a possible method of exposure to TCDD might be through soil contamination of the pelt during burrowing and movement of the animal through these burrows followed by subsequent ingestion of soil particles through grooming. The beach mouse was observed in the laboratory to spend much of its active period grooming. Thus, a laboratory study was conducted using alumina gel with and without 2.5 ppb TCDD to dust the pelts of test and control mice. This study confirmed that grooming is one method of uptake of TCDD by the beach mouse: levels of 45 to 89 ppt were found on the pelts and a level of 125 ppt was found in liver tissue.

The racerunner, Cnemidophorus sexlineatus, the most prevalent reptile on the grid, was also trapped and examined for gross defects as well as having tissue samples analyzed for TCDD residue. Significant levels of TCDD were found in the visceral mass (360 ppt) and the trunk (370 ppt) of reptiles collected from areas where soil levels of TCDD were the highest (Grid 1). These reptiles, however, showed no significant lesions or variations in visceral mass between control specimens and those collected from the contaminated sites.

A 1973 sweep net survey of the arthropods of the 1-square-mile test area resulted in the collection of 5966 specimens belonging to 71 insect families and two arachnid orders. These totals represent the collections from five paired sweeps taken over a 1-mile section of the test grid. A similar study performed in 1971 produced 1796 specimens, representing 70 insect families and one arachnid order, from five paired sweeps of the same area using the same basic sampling techniques. A much greater number of small to minute insects were taken in the 1973 study. Vegetative coverage of the test area had significantly increased since the 1971 study. Comparison of the results of the two studies also showed significant increases in the number of arthropod specimens and varieties per sampled grid transect, but there was little overall change in calculated community diversity. These results are not unexpected, and the population increases will continue as the test area stabilizes and develops further plant cover, thus allowing a succession of animal populations to invade the recovering habitat.

Species diversity studies were conducted in two aquatic ecosystems associated with TA C-52A (Trout and Mullet Creeks), while food chain studies were conducted only in one stream (Trout Creek) and a grid pond. Both the latter two locations were shown in 1973 to contain TCDD in silt. Since TCDD was not water soluble, a different route of entry into these aquatic systems was sought. Examination revealed that erosion of soil occurred into the pond on the test area and into the bayhead of Trout Creek immediately adjacent to Grid 1. TCDD levels of 10 to 35 ppt were found in silt of the aquatic systems, but only at the point where eroded soil entered the water. Species diversity studies of the stream were conducted in 1969, 1970, 1973, and 1974. Insect larvae, snails, diving beetles, crayfish, tadpoles, and major fish species from both aquatic systems were analyzed for TCDD. Species diversity studies indicated no significant change in the composition of ichthyofauna between these dates or a control stream. Concentrations of TCDD (12 ppt) were found in only two species of fish from the stream, Hotropis hypselopterus, sailfin shiner, and Gambusia affinis, mosquitofish. The sample of mosquitofish consisted of bodies with heads and tails removed. Two samples of sailfin shiners were analyzed; one containing viscera only and the other bodies less heads, viscera, and caudal fins. Only the viscera contained TCDD. Samples of skin, muscle, gonads, and gut were obtained from Lepomis punctatus, spotted sunfish, from the test grid pond. Levels of TCDD in those body parts were 4, 4, 18, and 85 ppt, respectively. Gross pathological observations of the sunfish revealed no significant lesions or abnormalities.

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PREFACE

This technical report represents the culmination and final report of an extensive three year cooperative research program with the Department of Life and Behavioral Sciences (now Department of Chemistry and Physiology), United States Air Force Academy, Colorado. The Air Force project directly related to the information in this report is Air Force Systems Command Project 74-5154-02, Ecological Survey of Test Area C-52A, Eglin Air Force Base, Florida. However, primary funding for the analysis of chemical residue in soils, plants, and animals, and the contractual expenses of many personnel were directly supported by the Air Force Logistics Command Project on the Disposition of the Herbicide Orange.

The assistance provided by the Armed Forces Institute of Pathology, the Air Force Environmental Health Laboratory, USAF School of Aerospace Medicine, USAF Regional Hospital/SGPE (Eglin Air Force Base), University of Alabama, and Colorado State University is gratefully acknowledged.

This report has been reviewed by the Information Office (OI) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Joe A. Farmer

JOE A. FARMER
Chief, Environics Office

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SECTION I

INTRODUCTION

In 1962, the Armament Development and Test Center, Eglin Air Force Base, Florida, was tasked with the responsibility for designing, developing, and testing aerial dissemination equipment in support of military defoliation operations in Southeast Asia. It was necessary for this equipment to be tested under controlled conditions that were as near to being realistic as possible. For this purpose an elaborate testing installation, designed to measure deposition parameters, was established on the Eglin Reservation with the place of direct aerial application restricted to an area approximately 1 mile square within Test Area C-52A (TA C-52A) in the southeastern part of the Reservation. Massive quantities of herbicide, used in the testing of aerial defoliation spray equipment from 1962 through 1970, were released and fell within the instrumented test area. The uniqueness of the area has prompted continued ecological surveys since 1967 (References 1 and 2). As a result, few ecosystems have been so well studied and documented.

A previous report (Reference 2) documented the persistence, degradation, and/or disappearance of the herbicides from the soils and drainage waters of TA C-52A, and the subsequent effects (direct or indirect) of the herbicides upon the vegetative faunal and microbial communities. The present report continues to document many of the studies previously reported in an attempt to expand or clarify earlier data. For some studies, e.g., vegetation and insects, qualitative and quantative data were collected in 1973, but complete analyses of the data were not available for the previous report. Studies on the rodent population, and in particular the beach mouse, Peromyscus polionotus, were expanded to include data collected during the summers of 1973 and 1974. These studies included histological examination and chemical analyses of tissues. In addition, a laboratory study was conducted to determine the probable route of uptake of the chemical contaminant 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The current study also examined the presence of a recovery phenomenon (noted in Reference 2) in streams draining the test area, and the presence of TCDD in the major food chains of the ichthyofauna of Trout Creek and a test grid pond.

1. DESCRIPTION OF GEOGRAPHICAL AND ENVIRONMENTAL FACTORS

a. General Area

The Eglin Air Force Base Reservation is located in Northwest Florida where it occupies a portion of Santa Rosa Island, Okaloosa Island, the southeastern part of Santa Rosa County, and the southwestern quarter of Walton County. It covers an area of approximately 750 square miles. To the south the Reservation is adjacent to Choctawhatchee Bay and the Gulf of Mexico, while to the north and east it is bordered roughly by the Yellow River and Alaqua Creek.

The Reservation lies on generally level or gently rolling terrain, all under 300 feet elevation and sloping to sea level on the west and south. It is drained by smaller streams that flow directly into Pensacola Bay and Choctawhatchee Bay. The valleys of these streams often are steep sided and terminate abruptly. The soil of most of the Reservation consists of somewhat excessively drained, deep acid sands of the Lakeland series. In the stream bottoms, and particularly along the Yellow River, the soils are much more heavily organic.

b. Test Area C-52A

Test Area C-52A (TA C-52A) is located in the southeastern part of the Eglin Reservation. It covers an area of approximately 3 square miles (Figure 1) and is a grassy plain surrounded by a forest stand that is dominated by longleaf pine (Pinus palustris), sand pine (Pinus clausa), and turkey oak (Quercus laevis). The actual area for test operations, which occupies an area of 2 square miles, is a cleared area occupied mainly by broomsedge (Andropogon virginicus), switchgrass (Panicum virgatum), and low growing grasses and herbs. Much of the center of the range was established prior to 1960, but the open range as it presently exists was developed in 1961 and 1962. Figure 2 is an aerial photograph of the 1-square-mile test grid and the immediate adjacent area as it appeared on 14 June 1973. The test grid is approximately 93 feet above sea level with a water table of 6 to 10 feet. The major portion of this test area is drained by five small creeks whose flow rates are influenced by a 61-inch average annual rainfall (Table 1). Data, by month, for 1973 and a norm (average for 9 years) are also given in Table 1 for temperature (minimum, maximum and average), wind (direction and speed), and relative humidiy (maximum and minimum). These data were recorded by an Automatic Meteorological Data Acquisition and Processing System (AMDAPS) unit with sensors located 6 feet above the soil surface and on a 300-foot tower in the center of the 1-square-mile test grid (Figure 1). For the most part, the soil of the test grid is a fine white sand on the surface changing to yellow sand beneath. The soils of the range are predominantly well drained, acid sands of the Lakeland Association with 0 to 3 percent slope. A typical 3-foot soil core would contain approximately 92 percent sand, 3.8 percent silt, and 4.2 percent clay with an organic matter content of 0.17 percent, an average pH of 5.6, and a cation exchange capacity of 0.8. A more extensive description of the soils associated with the test area is given in Reference 2.

2. DESCRIPTIONS OF SAMPLING GRIDS AND HERBICIDE DEPOSITION

Descriptions of the sampling grids located on TA C-52A and individual mission data including herbicide and total gallons sprayed have been compiled by Helen Biever (Reference 3), with a supplement by John Hunter (Reference 4). Test projects associated with this site were completed and the program terminated in December 1970.

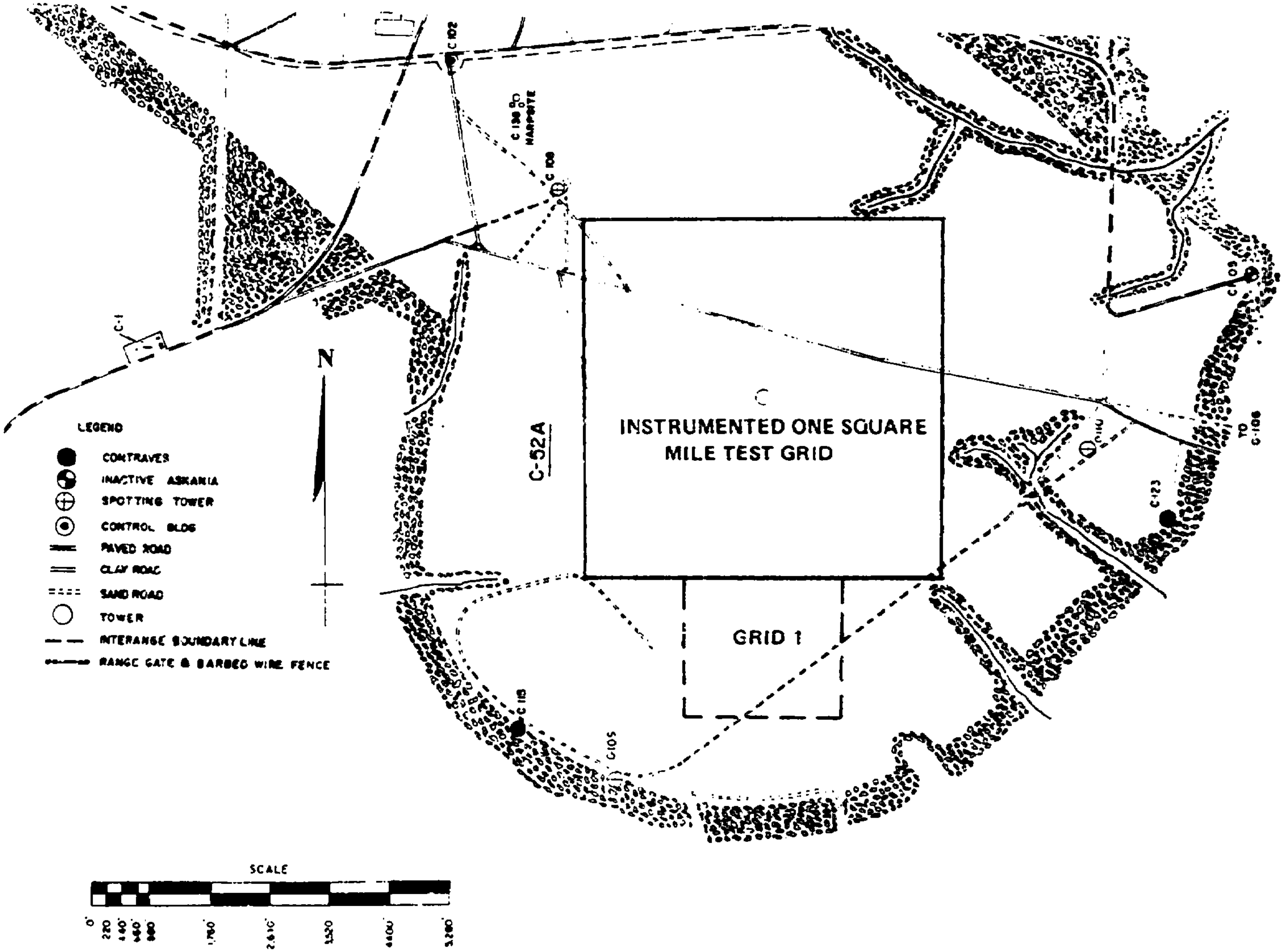

Figure 1. Map of Test Area C-52A, Eglin Air Force Base Reservation, Florida

Figure 2. Photograph of the 1-Square-Mile Grid Taken at 4300 Feet Above Ground Level on 14 June 1973

TABLE 1. MEAN MONTHLY WEATHER OBSERVATIONS FOR TEST AREA C-52A FOR 1973 VERSUS NORM (NINE YEARS OF DATA)[a]

MONTH	TEMPERATURE (°F) MIN	MAX	AVE	PRECIP (INCHES)	WIND DIRECTION	/SPEED	REL HUMIDITY MAX	MIN
Jan 1973:	42	60	51	4.51	N	7	87	50
Jan Norm:	42	61	52	4.02	N	7	84	50
Feb 1973:	40	60	52	3.41	N	7	84	44
Feb Norm:	45	64	53	3.68	N	7	82	45
Mar 1973:	56	67	63	12.25	SE	8	95	65
Mar Norm:	50	69	60	6.18	SE	8	81	42
Apr 1973:	56	72	66	7.89	WNW	8	90	52
Apr Norm:	57	76	67	5.00	S	7	84	44
May 1973:	66	80	74	3.98	SW	7	86	54
May Norm:	64	84	74	3.19	SW	7	88	54
Jun 1973:	72	87	80	3.13	SW	6	92	58
Jun Norm:	72	89	80	5.46	SW	6	94	57
Jul 1973:	76	90	83	8.71	SSW	7	94	61
Jul Norm:	74	90	83	7.58	SW	6	91	64
Aug 1973:	73	89	81	5.26	N	7	88	53
Aug Norm:	73	90	82	7.12	N	6	94	67
Sept 1973:	72	86	78	7.22	NE	6	90	61
Sept Norm:	70	87	78	7.68	NE	6	95	58
Oct 1973:	61	81	71	3.44	NE	8	88	49
Oct Norm:	59	80	70	2.63	N	6	89	56
Nov 1973:	55	73	64	3.30	N	6	91	61
Nov Norm:	48	69	59	3.57	N	7	84	53
Dec 1973:	42	62	52	8.02	NE	8	84	49
Dec Norm:	43	63	53	4.87	N	6	90	52
1973:	59.3	75.6	67.9	71.12		7.08	89.1	54.8
Norm:	58.1	76.8	67.6	60.98		6.58	88.0	53.5

[a]Sensors for wind and temperature were situated on the 300-foot tower at 12 and 6 feet above the soil surface, respectively.

a. Grid Descriptions

Figure 3 shows the location of the various herbicide grids that were located on TA C-52A. The original sampling grid (Grid 1) for spray equipment testing became operational in June 1962. It consisted of four intersecting straight lines in a circular pattern, each being at a 45-degree angle from those adjacent to it. This grid was discontinued after 2 years. It was located immediately south of the 1-square-mile grid.

The second sampling grid (Grid 2) consisted of three parallel lines intersected at right angles by another set of three parallel lines. These lines were 800 feet apart, thus forming four equal quadrants. The southwest corner of this grid corresponded to the southwest corner of the 1-square-mile grid. The parallel line grid was operational during the period May 1964 to November 1965.

The third sampling grid (Grid 3) consisted of three concentric circles, with respective diameters of 1200, 1600, and 2000 feet. This grid was located in the northeast quadrant of the 1-square-mile grid. The concentric circles grid was operational between October 1967 and April 1968; however, difficulty in interpreting data from this sampling array caused use of this grid to be discontinued.

The fourth sampling grid (Grid 4) was the 1-square-mile grid, the center of which was marked by the 300-foot tower. This was the last testing grid used on TA C-52A and its inwind and crosswind sampling arrays extended into Grid 2 and Grid 3. The two inwind and four crosswind sampling arrays of Grid 4 became operational in May 1968. Each inwind array consisted of three parallel rows spaced 400 feet apart, with 297 sampling stations per row. The aircraft flight path crossed the midpoints of the sampling lines. The crosswind sampling arrays consisted of three parallel rows 400 feet apart, with 253 sampling stations per row.

The complex of defoliant grids, intersecting near the central AMDAPS tower and oriented to eight major compass headings, provided 16 discrete sampling grids which could be selected for the most advantageous wind conditions prior to and during mission time. Glass plates and Kromekote cards were employed for physical collection of test materials in droplet form. Each of 250 permanent sampling stations of the TA C-52A basic grid array were equipped with a wide variety of sampling devices, including the above, but were also equipped with individual commercial power and sequencing control lines for remote operation of automatic vacuum type samples which collected small particles and aerosol test materials. These sampling stations were arranged on 400-foot centers to form the 1-square-mile grid (see sampling station array in Figure 4). Remotely controlled, battery operated, portable samplers were also available to gather data in special purpose grid configurations anywhere in a 10-square-mile area.

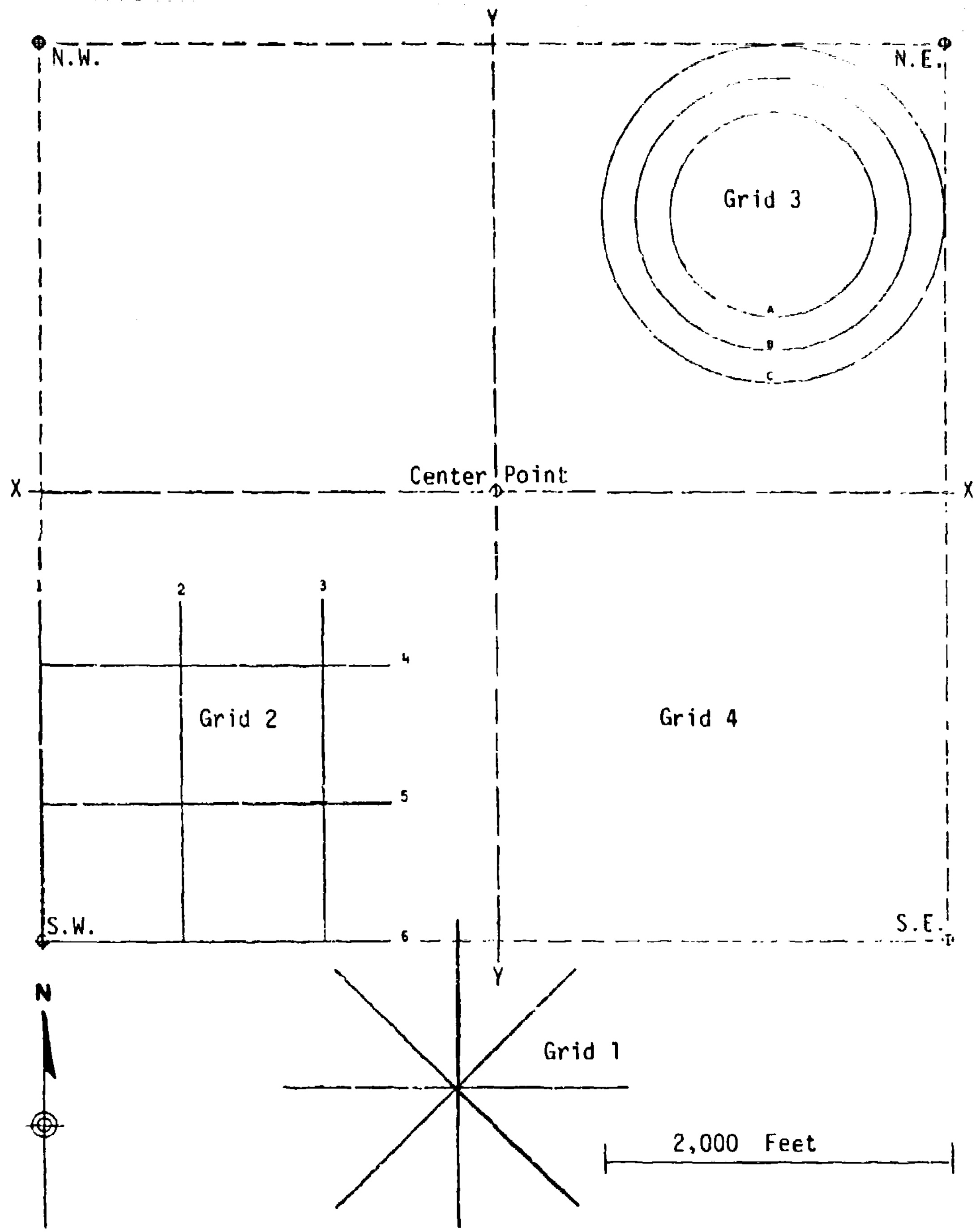

Figure 3. Location of Test Grids Used During Dissemination of Herbicides Over Test Area C-52A

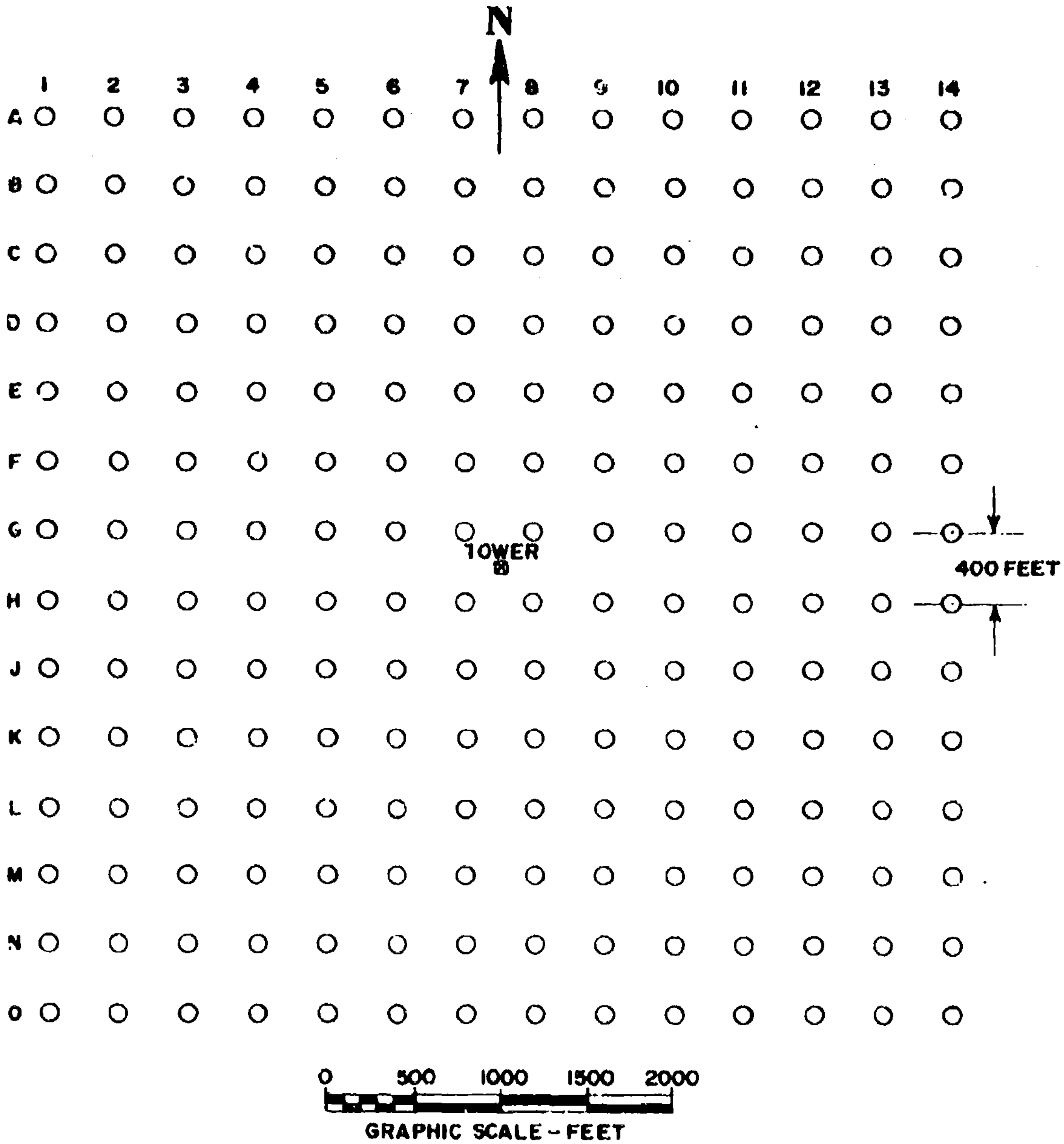

Figure 4. Location of the Permanent Sampling Stations on the 1-Square-Mile Grid

b. Deposition Rate

Herbicide formulations, deposition rates and dates, and locations of major and minor flight paths for the 1-square-mile testing grid on TA C-52A are given in Figure 5[(1)]. Despite excellent records as to the number of missions and quantity of herbicide per mission, there was no way to determine the exact quantity of herbicide deposited at any point or on any one of the instrumented grids (Grids 2, 3, and 4). Figure 5 also shows the quantity of herbicide deposited on the non-instrumented grid (Grid 1, 1962-64) immediately south of the 1-square-mile instrumented grid (see also Figure 3). Grid 1 received nearly 1900 pounds per acre of the herbicides 2,4,5-T and 2,4-D (2,4-dichlorophenoxy-acetic acid). (For additional information on annual dissemination of the herbicides and the description of chemical formulations see Reference 2.)

Footnote

(1)Figure 5 reprinted from Bartleson, F. D., D. D. Harrison, and C. I. Miller: A Survey of Trees on a Herbicide Treated Test Area, Eglin AFB, Florida. AFATL-TR-74-190, Air Force Armament Laboratory, Eglin Air Force Base, Florida, 1974.

TOTAL NUMBER OF POUNDS OF HERBICIDE

Years	2, 4-D	2, 4, 5-T	Picloram	Cacodyloc Acid	Arsenic
1968-1970	44,010	38,450	1,501	12,595	1,889
1966-1970	2,784	------	752	1,029	154
1964-1966	35,026	35,026	--------	---------------	------
1962-1964*	87,186	87,186	--------	---------------	-------

*Center of flight paths during this period was located approximately 1,000 feet south of marker N-7.

Solid Lines - Major Flight paths

Dashed Lines - Minor Flight paths

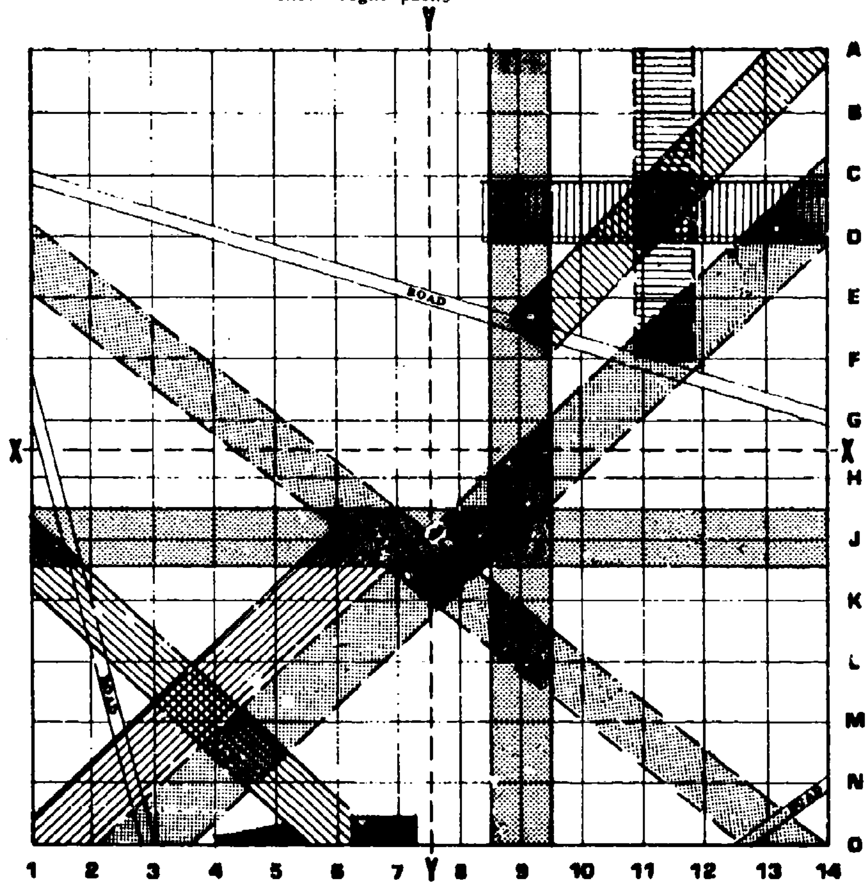

Figure 5. Flight Paths Used for the Dissemination of Herbicides on the 1-Square-Mile Grid of TA C-52A (Major Flight Paths Used are Shown with Solid Lines and Minor Flight Paths by Broken Lines)

SECTION II

VEGETATIVE ASSESSMENT OF TEST AREA C-52A

Because of the massive amounts of plant control chemicals (herbicides) applied to Test Area C-52, a unique opportunity has existed to evaluate changes in vegetative patterns and/or succession. Eight previous studies, although concerned with the vegetation on or adjacent to the test area, have eluded primarily to qualitative changes in vegetation. This report documents with quantitative data the actual composition of the plant communities on Test Area C-52 and attempts to place these data in perspective with the previous studies.

1. SYNOPSIS OF PREVIOUS VEGETATIVE STUDIES

In 1967, Ward (Reference 1) studied the plants adjacent to the test area in order to determine the effects, if any, of the testing program on vegetation surrounding the test area. Turkey oaks immediately adjacent to the test area had been severely damaged by defoliant drift, but little if any damage was noticeable on longleaf or sand pines. Ward observed the dominant vegetation in all directions from the test area, but was unable to find any damage other than that caused by the 1962-1964 period of equipment testing. In 1968, Ward (Reference 5) collected and identified 54 species of plants occurring on the instrumented 1-square-mile grid. At this time the area was receiving repetitive application of 2,4-D, 2,4,5-T, and picloram herbicides. Nevertheless, Ward noted that the majority of the different plant species occurring on the test grid were broadleaf plants (dicotyledenous plants, herbicide sensitive). However, the bulk of the plant cover was composed of grasses and yucca (monocotyledenous plants, herbicide resistant). A final report by Ward in 1970 documented plant communities and plant species occurring on the Eglin Reservation. Hunter and Agerton (Reference 6) measured the annual growth rings of selected trees (sand pine and longleaf pine) adjacent to the test area, but did not find a relationship between annual defoliant dissemination and annual growth rings. In 1970, Sturrock and Young (Reference 7) conducted an histological study on crown tissue of yucca (Yucca filamentosa) found growing in the soil of the grid that contained detectable (ppm) levels of herbicide residue. A gross comparison of control plants and those on the grid showed differences; however, an histological examination revealed no differences. Both samples followed the normal structural development described for yucca in the scientific literature.

Nine months after the last defoliant-equipment test mission (June 1971) on the 1-square-mile grid of Test Area C-52A, Hunter and Young (Reference 8) conducted the first detailed vegetative cover survey. Since the 1-square-mile grid has 169 permanent air sampling stations (Figure 4), these stations and their interconnecting clay-covered access roads provided the boundaries for dividing the grid into 169 sections (each 400 by 400 feet). Within each section the percentage vegetative coverage was ranked as Class 0, 0 to 5 percent; Class I, 5 to 20 percent; Class II, 20 to 40 percent; Class III, 40 to 60 percent; Class IV, 60 to 80 percent; and Class V, 80 to 100 percent coverage. Sections were designated by the number of the permanent sampler in the northwest corner of the section, e.g., B-8 and J-4. Three of the 400-by-400-foot sections within each coverage class were selected at random and a diagonal transect starting 20 feet within the northwest corner of each section was walked to the southeastern boundary. All dicotyledonous (broadleaf) plants were collected along the transect. Data were tabulated for the number of dicotyledonous plants occurring in each section. Then, one of the 400-by-400-foot sections from each class were randomly selected for further study and identification of plants. A control area 0.2 mile northwest of the 1-square-mile grid and the center of the area formerly occupied by Grid 1 were also surveyed.

Most of the plants collected during this study were permanently mounted and added to the Eglin Reservation Herbarium. A photographic record of the vegetation coverage of the test grid was initiated to serve as a baseline for future observations.

Seventy-four dicotyledonous species were found on the 1-square-mile grid and the average number of species within vegetative coverage classes ranged from five in Class 0 (0 to 5 percent cover) to 24 in Class V (80 to 100 percent cover). A control area contained 28 species, 26 of which were also on the grid. Diodia teres, Rhynchosia galactioides, and Tithymalus spaerospermus were the most common dicotyledons and D. teres, T. spaerospermus, Stylism villosa, and Lupinus diffusus were the first plants to invade barren or Class 0 areas. The existence in Class 0 or Class I areas of dicotyledonous plants that were susceptible to the active ingredients of military defoliants indicated that the amount of defoliant residues in the soil was insignificant. It was suggested that vegetative coverage of sections of the grid was due to a combination of soil moisture content, prior mechanical disturbances and prior defoliant spraying. Although vegetation was removed by defoliant spraying in areas under flight paths, the low level of vegetative coverage in other areas was primarily due to prior mechanical clearing. In those areas having a relatively high soil moisture content, considerable vegetation had returned, regardless of whether the vegetation was removed mechanically or by defoliants.

In 1973, another vegetation assessment of Test Area C-52A was conducted (Reference 2). As in the June 1971 study, three 400-by-400-foot sections from each coverage class were randomly selected for a detailed collection of dicotyledonous (broadleaf) plant species. A diagonal transect starting 20 feet within the northwest corner of each section was walked to the southeastern boundary. Plants were collected along the transect, and the results were tabulated for the number of dicotyledonous plants occurring in each section. A control area 0.2 mile northwest of the 1-square-mile grid and an area in the center of the plot formerly occupied by Grid 1 were also surveyed. A square-foot analysis was performed on (1) 15 additional 400-by-400-foot sections, (2) the control area used in 1971, (3) a new control area west of the 1-square-mile grid, and (4) a 160,000-square-foot section in the center of the area occupied by Grid 1. The additional 15 sections were randomly selected, and within each section, nine areas, each measuring 1 square foot, were analyzed (Figure 6). Percent coverage for each plant species within the square foot was visually estimated.

Significant changes in vegetative coverage were noted from 1971 to 1973 (Table 2). The greatest percent change occurred in those areas with the smallest amount of vegetation. Moreover, 33 additional plant species were found during the June 1973 survey that were not collected in May 1971. A detailed analysis of data from the square-foot survey was not included in the 1973 report.

The most recent study of vegetation on Test Area C-52A concerned a survey of trees (Reference 9). Between June and August 1974, 5155 trees were counted in the 845 sample plots on the 1-square-mile grid of TA C-52A, representing an average of 126.9 trees per acre. The average was misleading, however, because 66 percent of the sample plots contained no trees. The dominant species were live oak (*Quercus virginiana* Mill) and turkey oak (*Quercus laevis* Walt). Five other species of oaks (*Quercus* spp), three species of pines (*Pinus* spp), and the common persimmon (*Diospyros virginiana* L.) were also found in the sample plots.

The mean height for the 5155 trees counted was less than 2 feet. Only 41 of these had a height over 6 feet, the tallest being 11 feet. Most of the trees were oaks found in small but dense clusters originating from the roots of previous trees. Trees starting from seeds, such as pines, persimmons, and single oaks, were relatively sparse, but their presence indicated the area was recovering.

Although the data were not statistically analyzed, there did appear to be some correlation between previous spray aircraft flight paths and plots with no trees. There also appeared to be some correlation between the presence of trees and the damper Chipley and Rutledge sands, as well as the proximity to the two major clay roads which cross the grid.

Many of the eight reports reviewed above elluded to vegetative succession, ecological recovery, and relative frequency of occurrence of plant species. However, with the exception of the reports on use of the square-foot analysis technique (Reference 9), and the survey of trees, quantitative information is lacking. Unfortunately, the report which discussed the use of the square-foot analysis technique did not include an in-depth analysis of the data obtained. Furthermore, the report on the tree survey was confined to observations on just tree species.

The purposes of the present report are to (1) present an in-depth analysis of the data obtained by the use of the square-foot technique employed for the vegetative survey in June 1973, and (2) relate these data to information obtained from previous studies in an attempt to understand the vegetative changes that have occurred on this unique area.

Figure 6. Square-Foot Analysis Technique Used During Vegetative Survey, June 1973

TABLE 2. PERCENT OF VEGETATION COVER OCCUPIED BY VEGETATIVE CLASS FOR THE 1-SQUARE-MILE GRID

VEGETATIVE CLASS	JUNE 1971 (Percent)	JUNE 1973 (Percent)
0 (0 to 5 percent)	4	0
I (5 to 20 percent)	14	4
II (20 to 40 percent)	29	12
III (40 to 60 percent)	25	18
IV (60 to 80 percent)	21	42
V (80 to 100 percent)	4	23

2. ANALYSIS OF SQUARE-FOOT QUADRAT DATA

a. Discussion of the Sampling Procedure

Whenever an ecological community is sampled, the data consist of lists of species present in each sample unit and number of individuals of a given species in each sample unit. Normally, sample units are areas or quadrats of a specified size. Since the major grid on Test Area C-52A (Grid 4) is a 1-square-mile grid that is subdivided into 169 sections, each 400 by 400 feet (Figure 3), these sections should be ideal. However, when arbitrarily delimited quadrats are used, there is always the risk that the classification obtained may be markedly affected by quadrat size and, in this case, previous history. The location of flight paths used for disseminations tests, the location of water (ponds and drainage ditches), and the change in soil type all have influenced the vegetative composition of the test grid.

The quantity of each species can be obtained by determining the ground cover occupied by a species. Ground cover may be defined as the area of ground occupied by a perpendicular projection onto it of the foliage and stems of individuals of a particular species. Cover values are usually expressed as percentage figures and may either be estimated or measured. Estimations are in common usage in broad surveys and usually appear in the form of cover-abundance data. Abundance and cover relate, then, to the floristic composition of the vegetation and are basically estimates of the number of individuals of the component species in a vegetation type.

The square-foot (quadrat) analysis method consists of randomly sampling a given number of square feet of surface (e.g., nine) within an homogeneous area of vegetation. An estimate of the ground cover by individuals is then recorded. Percentage cover measured in this manner is a good method for calculation of species abundance, particularly in a grassland vegetation, i.e., a type where large scale layering is minimal and where a detailed analysis of herbage (broadleaf) composition is of applied value. There are three main drawbacks to the use of this method: (1) the need to randomize the placing of the square foot within the homogeneous area of vegetation, (2) its use is restricted to low, unlayered vegetation types, and (3) there is a tendency to exaggerate the estimate of percentage cover, hence, it is important to maintain a proper frame of reference (e.g., dividing the square foot into quarters of 25 percent units when making the estimates.

b. Methods and Materials

In preparation for the square-foot analysis, all 169 400-by-400-foot sections were visually rated for vegetative cover. Each section was placed in one of five classes: 5 to 20 percent, 20 to 40 percent, 40 to 60 percent, and 80 to 100 percent, for Class I through Class V, respectively. Three sections from each vegetative class were randomly selected for the analysis. The actual square-foot sampling sites were selected by dividing the 400-by-400-foot sections into three strips, each 133 feet wide. A line was drawn in the center of each strip and three 1-square-foot areas were selected by generating d_1, d_2, and d_3 distances to be walked. The distances were generated by a random number generator which included constraints that assured one sampling area from each one-third of the traverse with the sampling area being random within the one-third distance. After each distance was walked off, the metal square foot was placed, and the percent coverage for each plant species within the square foot was visually estimated.

c. Results

Photographic records from various locations on TA C-52A confirmed the rapidity of vegetative succession. Figures 7 and 8 show the continued succession of plants onto Grid 1 (photograph taken from Sampler Station 0-9). It was apparent that the various grass species dominated the bulk of the new biomass. Closer examination of the new plant community indicated that large seeded grasses, e.g., Panicum lanuginosum, wooly panicum, and Panicum virgatum, switchgrass, dominated the previously denuded areas (Figures 9 and 10). Photographic records show that with longer periods of time the invasion of dicotyledonous annuals and other perennial grasses also occurred. Figure 11 shows the sequence of vegetative succession on Grid 1, beginning in 1964 after the last herbicide application on this site; again in late spring 1969 with the grass dominated community; and finally in mid-1974 with herbaceous dicots, grasses, and small shrubs found throughout the area.

Data from the analysis of square-foot quadrats supported the photographic records. With each new season, the areas previously denuded by repetitive applications of herbicide changed in the amount of vegetative cover (increased). This change was both visually estimated and quantitatively measured. Table 3 gives a comparison of the vegetative classes and the control and Grid 1 areas. Visual estimates were always higher for every site than those determined by the square-foot quadrat.

As noted, the method of sampling (square-foot quadrats) provided data on both ground cover and species abundance for each vegetative class. Species abundance related to the number of times a given species was found (intercepted) when the square foot was randomly placed within the area to be sampled. The data in Table 4 indicated that dicots were intercepted more frequently than monocots (grasses) and that this trend was followed with the increase in vegetative class. Undoubtedly, the increase in number of different dicot species accounted for the increase in mean intercepts. Table 5 shows the vegetative density of the 13 major (dominant) plant species occurring on the 1-square-mile grid. Note that wooly panicum, rough buttonweed (Diodia teres), switchgrass, and broomsedge (Andropogon virginicus) were found in every vegetative class. With increase in ground cover, more species were found. Class IV sites (60 to 80 percent ground cover) and Class V sites (80 to 100 percent ground cover) contained species not found in less dense areas, e.g., Gnaphalium obtusifolium (fragrant cudweed), Erechtites hieracifolia (fireweed), and Sisyrinchium rufipes (blue-eyed grass). Note that of the 13 species considered dominant in ground cover, six of these were grasses. Table 6 compares the

Figure 7. October 1971: Looking South
Near Sampler 0-9

Figure 8. August 1974: Looking South
Near Sampler 0-9

Figure 9. Seeds of Major Grass Species at Time of Maturity (Late Fall)

Figure 10. Typical Grass-Dominated Seral Stage Three Years After Seed Invasion

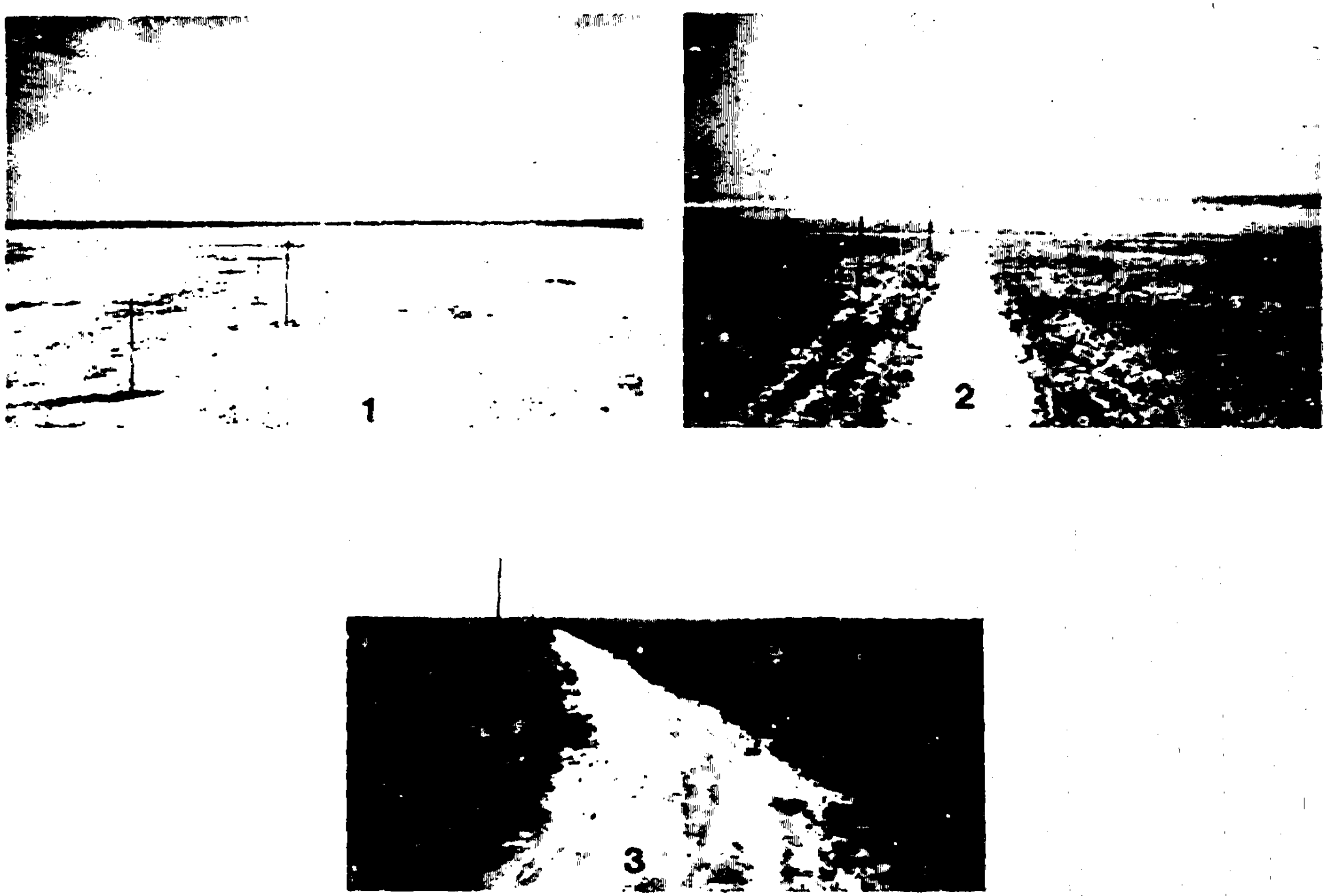

Figure 11. Sequential Seral Stages of Plant Succession on Grid 1, Test Area C-52A:
1. Area Immediately Following Last Herbicide Application, May 1964;
2. Grass-Dominated Seral Stage, April 1969;
3. Forb-Grass-Seedling Shrub Stage, June 1964.

TABLE 3. DATA COMPARISON OF PERCENT VEGETATIVE COVER BY VISUAL OBSERVATION OF OVERALL PLOT VERSUS THE SQUARE-FOOT TRANSECT METHOD, 1973 DATA

VEGETATIVE CLASS/SITE	VEGETATIVE CLASS CRITERIA	VISUAL ESTIMATE[a]	SQUARE-FOOT TRANSECT[b]
Class I	5 to 20	19	11
Class II	20 to 40	29	19
Class III	40 to 40	60	41
Class IV	60 to 80	76	67
Class V	80 to 100	89	80
Control Site 2[c]		75	45
Grid 1[d]		75	47

[a]Average of 12 estimates

[b]Average of 27 transects

[c]Located 0.1 mile west of Sampler E-1

[d]Center section of Grid 1 located 1000 feet south of Sampler 0-7

TABLE 4. INTERCEPT DATA FOR EACH VEGETATIVE CLASS, GRID 1, AND CONTROL SITE VIA SQUARE-FOOT TRANSECT METHOD, 1973 DATA

VEGETATIVE CLASS	MEAN INTERCEPTS PER 9 SQUARE FEET		MEAN NUMBER OF SPECIES ENCOUNTERED	
	DICOTS	MONOCOTS	DICOTS	MONOCOTS
Class I	7.3	7.3	6	8
Class II	7.0	9.0	8	5
Class III	18.7	16.3	17	10
Class IV	22.3	19.0	18	8
Class V	25.7	17.0	20	8
Control	16.0	5.0	40*	14[a]
Grid I	8.0	5.0	19*	20[a]

[a]Number of species encountered per 27 square feet of transects

TABLE 5. VEGETATIVE DENSITY (PERCENT GROUND COVER) OF THE DOMINANT PLANT SPECIES OCCURRING ON TEST AREA C-52A

SPECIES	CLASS I			CLASS II			CLASS III			CLASS IV			CLASS V		
	N-9	N-5	M-12	B-9	L-5	N-10	L-13	D-2	K-7	C-11	K-11	I-4	B-4	E-6	F-12
Panicum lanuginosum	5.79	2.39	2.78	17.00	7.11	3.44	0.33	9.00	11.33	20.77	0.33	22.00	3.67	4.11	[illegible]
Diodia teres	1.79	1.89	2.00	0.75	0.94	2.06	2.38	2.00	3.67	--	1.22	3.33	1.10	4.59	--
Panicum virgatum	0.06	5.50	4.11	2.22	0.55	4.06	6.00	18.78	9.67	27.33	26.22	2.00	37.78	33.67	14.11
Hypericum gentianoides	--	0.11	--	--	--	--	1.33	[illegible]	2.67	0.67	2.11	2.59	0.33	3.33	0.22
Gnaphalium obtusifolium	--	--	--	--	--	--	0.22	0.77	--	0.44	4.11	0.78	5.11	1.33	0.44
Paspalum setaceum	--	--	--	--	--	0.17	2.44	2.22	0.22	2.33	7.78	12.39	--	5.22	--
Eragrostis refracta	0.94	--	0.33	0.67	--	1.72	0.61	7.11	5.33	0.56	8.39	0.44	--	--	--
Paronychia [illegible]	--	--	--	--	--	--	0.99	--	1.56	0.22	0.78	1.78	0.22	2.11	0.56
Andropogon virginicus	--	--	0.33	0.89	4.44	--	1.67	--	1.56	14.00	--	3.33	[illegible]	--	24.22
Polypremum procumbens	--	--	--	1.11	--	--	--	0.22	--	0.11	--	0.67	3.56	1.11	[illegible]
Erechtites hieracifolia	--	--	--	--	--	--	0.16	--	0.22	0.44	0.59	0.67	1.33	[illegible]	--
Rumex acetosella	--	--	--	--	--	0.56	0.94	[illegible]	0.77	[illegible]	--	0.22	--	0.33	--
Sisyrinchium [illegible]	--	--	--	--	--	--	--	--	--	--	[illegible]	0.44	--	[illegible]	24.56

TABLE 6. FREQUENCY (NUMBER OF SQUARE-FOOT TRANSECTS INTERCEPTED) OF MONOCOTYLEDONOUS SPECIES (GRASSES) ENCOUNTERED IN THE 1973 SURVEY OF THE 1-SQUARE-MILE GRID ON TEST AREA C-52A

SPECIES	CLASS I			CLASS II			CLASS III			CLASS IV			CLASS V			TOTAL
	N-9	N-5	M-12	B-9	L-5	N-10	L-13	D-2	K-7	C-11	K-11	E-4	B-4	E-6	F-10	
Panicum virgatum	1	2	2	1	1	2	2	4	3	4	4	4	6	8	5	49
Panicum lanuginosum	2	2	3	8	4	3	1	6	5	7	1	7	4	3	5	61
Eragrostis refracta	3	--	2	2	--	3	1	4	3	1	2	1	--	--	--	22
Panicum angustifolia	1	--	--	--	--	--	1	--	--	--	1	--	--	--	--	3
Paspalum notatum	1	--	--	--	--	--	1	--	1	--	--	1	--	--	--	4
Andropogon virginicus	--	--	1	1	1	--	1	--	1	4	--	1	1	--	7	18
Bulbostylis [illegible]rei	--	--	1	--	--	--	3	--	--	--	--	--	1	--	--	5
Rhynchospora tra[illegible]	--	--	1	--	--	--	--	--	--	--	--	--	--	--	--	1
Paspalum setaceum	--	--	--	--	--	1	3	1	1	6	2	7	--	[illegible]	--	24
Aristida stricta	--	--	--	--	--	--	4	--	--	--	--	--	--	--	--	4
Eremochloa ophiuroides	--	--	--	--	--	--	--	--	3	--	--	--	--	--	--	3
Sisyrinchium rufipes	--	--	--	--	--	--	--	--	--	--	1	3	--	2	4	10
Arundinaria tecta	--	--	--	--	--	--	--	--	--	--	--	--	1	--	--	1
Panicum ashei	--	--	--	--	--	--	--	--	--	--	--	--	--	--	1	1

frequency of interception for the grass species found on the 1-square-mile grid. In frequency of abundance, the major species were wooly panicum, switchgrass, thin paspalum (Paspalum setaceum), coastal lovegrass (Eragrostis refracta), and broomsedge. Although thin paspalum was frequently encountered, it was so only in Class III and IV sites.

From the above data on ground cover and abundance, a general pattern of vegetative succession could be established. The large seeded grasses, switchgrass and wooly panicum, were the first perennial species to assume dominance. The annual herbs, rough buttonweed and poverty weed (Hypericum gentianoides), spread radidly into the loose soil around and between the dominant grasses. With time, other species occupied significant vegetative cover, especially broomsedge, coastal lovegrass, fireweed, and fragrant cudweed. In general, areas having 5 to 20 percent vegetative cover contained 14 species (eight grasses and six herbs) while an area of 80 to 100 percent cover contained 28 species (eight grasses and 20 different herbs).

Trends in vegetative succession are most influenced by climatic factors, thus an analysis of climatic data for TA C-52A was necessary.

d. Climatic Studies

Variations in climate can exist in a fairly small geographical area, and such variations can be seen across the Eglin Air Force Base Reservation. Because the testing of spray equipment necessitated accurate knowledge of weather conditions, Test Area C-52A was endowed with an especially fine data base for the three meteorological parameters of temperature, wind, and dewpoint (Reference 10). During the major years of testing spray equipment, 1963 through 1969, the Automatic Meteorological Data Acquisition and Processing System (AMDAPS) was in operation at this site, automatically collecting and processing data from meteorological sensors on a series of towers. Sensors for winds and temperatures were situated on a central 300-foot tower at the surface (12 feet for wind, 6 feet for temperature), and at 54, 162, and 300 feet AGL. A dewpoint sensor was also located at the 12-foot level.

At the four corners of the 1-square-mile grid, centered on the 300-foot tower, were four low towers measuring the wind at the 12-foot level. AMDAPS measured, processed, and stored data from the sensors at 1/2 second intervals, smoothed over a 15-second period, integrated over a 5-minute interval, and means and standard deviation printed out every 15 minutes.

Figure 12 shows the variation by time of day of the monthly mean surface wind quadrant. The sea breeze effect can be seen quite clearly in the swinging of the wind from north around to east and south. The earlier advent of the sea breeze with advancing season is also well defined. Wind speeds are similarly shown in Figure 13. Mean surface temperatures for the same time periods are shown in Figure 14.

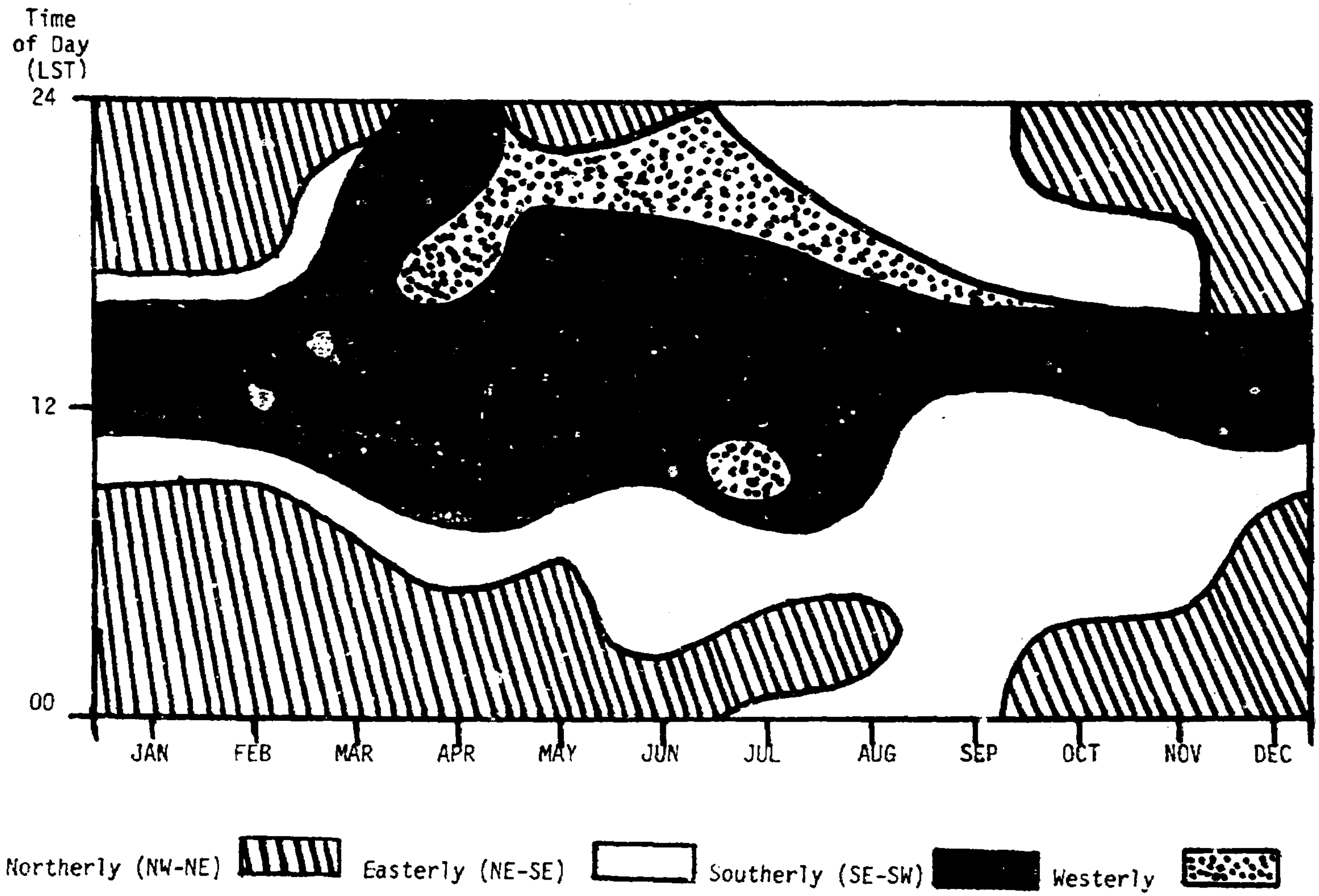

Figure 12. Monthly Mean Surface Wind Quadrant, Test Area C-52, by Time of Day[a]

[a]Data were recorded by the AMDAPS (Automatic Meteorological Data Acquisition and Processing System) at Site C-52; with 1/2 second sensing interval, 15-second smoothing, 5-minute integration, 15-minute averaged printout. Period of record is January 1963 - October 1969.

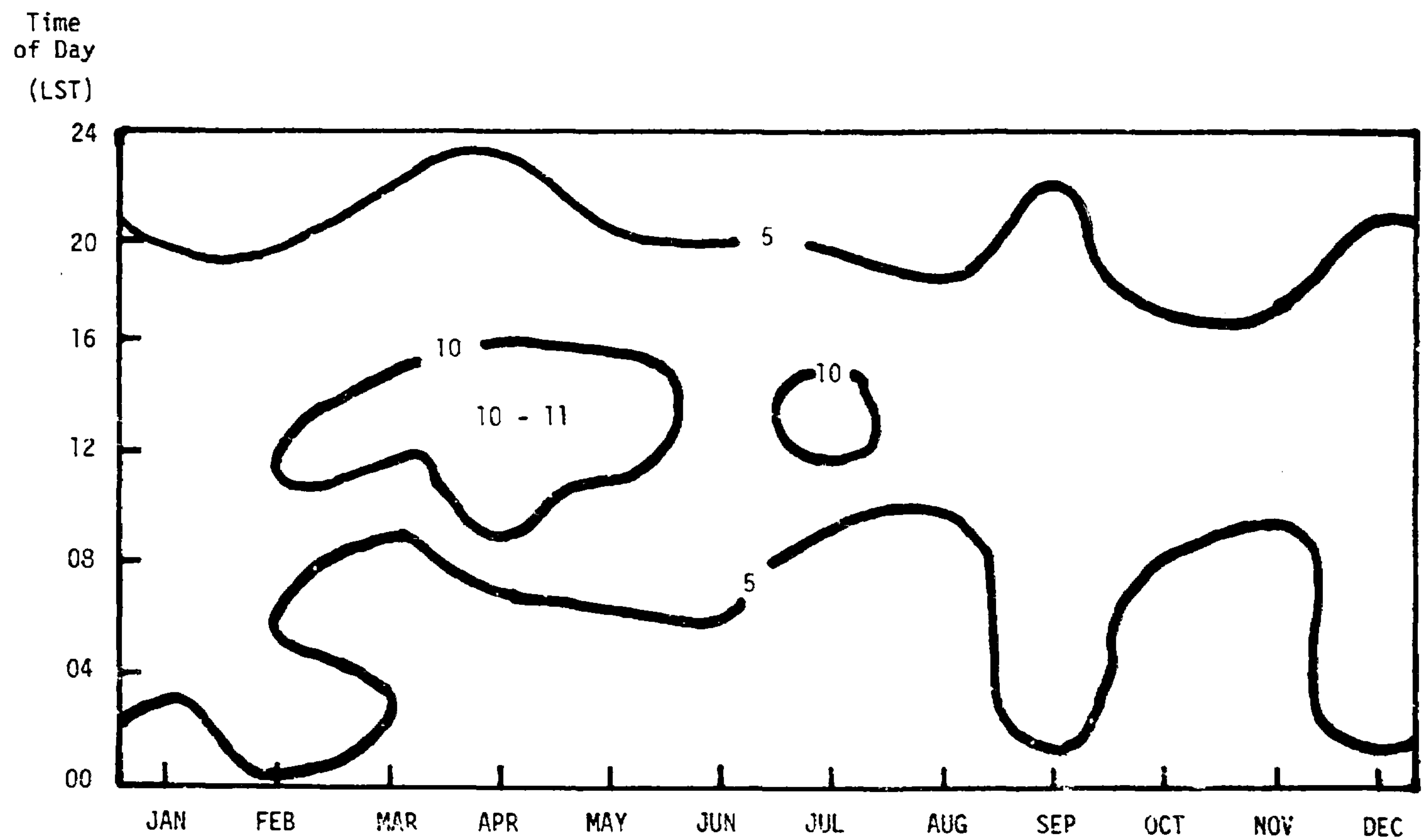

Figure 13. Monthly Mean Surface Wind Speed, Test Area C-52, by Time of Day[a]

[a]Wind speed shown in knots. Data were recorded by the AMDAPS at Site C-52; with 1/2 second sensing interval, 15-second smoothing, 5-minute integration, 15-minute averaged printout. Period of record is January 1963 - October 1969.

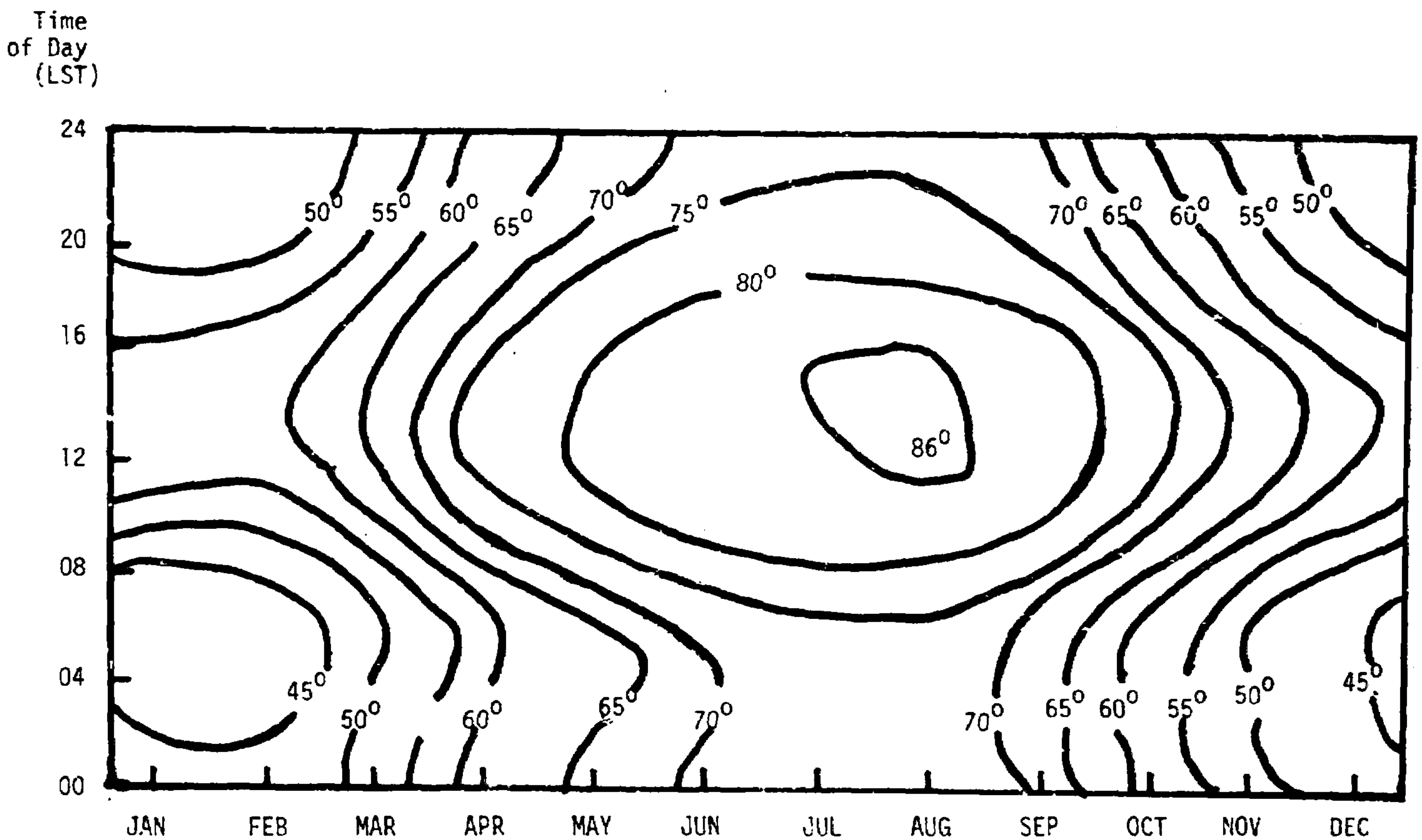

Figure 14. Monthly Mean Surface Temperature, Test Area C-52, by Time of Day[a]

[a]Temperature shown in degrees Fahrenheit. Data were recorded by the AMDAPS at Site C-52; with 1/2 second sensing interval, 15-second smoothing, 5-minute integration, 15-minute average printout. Period of record is January 1963 - October 1969.

3. DISCUSSION

Earlier technical reports (References 2 and 8) indicated that vegetative succession was most rapid in the northern portion of the 1-square-mile grid, e.g., areas near A-9 and C-11, and least rapid in the southern portion, e.g., near areas N-9, 0-7 and the northern section of Grid 1 (Figures 3 and 4). This lack of vegetative cover in the southern section of the test area prompted Vitro Services, Vitro Corporation of America (prime contractor during the operational stages of the spray equipment testing program), in the mid-1960s to seed thin paspalum (Paspalum setaceum), bahiagrass paspalum (Paspalum notatum), and common centipede grass (Eremochloa ophiuroides). These species were established but have generally remained confined to the areas where they were originally planted, e.g., K-7, L-13, and N-10.

In the late summer of 1973, Robert Sayers conducted a study of seed dispersal and regeneration potential of the major grass species on the test area, e.g., woolly panicum and switchgrass. He found that although abundant seed was produced in the central section of the test area, the bulk of the seed was dispersed in a northward direction because of prevailing winds at the time of seed drop. Moreover, those grass and herbaceous seeds dispersed into the southern section of the test area could not establish themselves in the moving sands created by a slightly higher topography beginning near N-5 and M-6 and extending into Grid 1.

Data collected on Grid 1 in support of the animal studies (Section III) indicated that the beach mouse (Peromyscus polionotus) favored areas of low vegetative cover. Analysis of the diet of these animals through examination of burrow and stomach contents, indicated that the large-seeded grasses were of prime importance in their survival. These same large-seeded grasses are those associated with the early stages of vegetative succession. The data suggest that rodents may act as biological dispersal agents into areas of low vegetative cover.

The vegetative studies have suggested that the areas which received repetitive applications of herbicides have and will continue to revegetate. However, because of introduced species these sites will never be the same as the control sites. Many areas of the test grid have already exceeded the vegetative density of the control sites. The effects of brush and young trees have, so far, been minor. However, all of the cleared area of Test Area C-52A may once again return to a Pinus (pine tree)-dominant plant community unless checked by the use of mechanical and/or chemical methods of brush control.

SECTION III

ANIMAL STUDIES OF TEST AREA C-52A

The first in-depth animal survey was conducted on the herbicide equipment test grid and surrounding area between May and October of 1970 (Reference 11). This survey was conducted while aerial spray equipment was actively being tested with the purpose of determining species variation and distribution patterns of the grid and surrounding 11 square miles. Of the 86 species of vertebrate animals observed or collected it was concluded that the beach mouse (*Peromyscus polionotus*) and the six-lined racerunner (*Cnemidophorus sexlineatus*) were present in numbers suitable for future population studies.

1. SYNOPSIS OF 1973 STUDY

Based on the information found in Reference 11 a two-fold study was initiated in 1973 (Reference 2). The purposes were (1) to obtain animals for examination of gross and microscopic lesions, since it had been reported (Reference 12) that TCDD produces teratogenic and embryotoxic effects under certain experimental conditions, and (2) a trapping survey was conducted to correlate habitat preference of the beach mouse on the grid in order to determine if population distribution was related to vegetative cover.

During the first part of the 1973 study several species of animals were caught, both on and off the test grid. The animals caught in significant numbers included the beach mouse, hispid cotton rats (*Sigmodon hispidus*), and six-lined racerunners (Table 7).

Gross necropsy lesions were relatively infrequent in all of these animals and when present consisted primarily of lung congestion in those animals that had died prior to being brought into the laboratory (Figure 15).

Histologically, the tissues of over one-half of the animals were considered completely normal. Microscopic lesions were noted in some animals from both groups. For the most part, these were minor changes of a type to be expected in any animal population. The most common findings were parasite infestations (Figures 16 and 17). Parasites may be observed in any species, and those present in this population were for the most part incidental findings that were apparently not harmful to the animal.

Several of the captured female beach mice were pregnant. The stage of gestation varied considerably from early pregnancy to near term. Fifty-four embryos and fetuses were examined grossly and microscopically. No developmental defects or other lesions were seen in them and no developmental defects were seen in any of the adults.

TABLE 7. TOTAL NUMBER AND LOCATION OF ANIMALS COLLECTED DURING PART I OF THE 1973 TEST PROGRAM

COMMON NAME	CONTROL	TEST	TOTAL
Beach Mouse			
Male	5	26	31
Female	5	18	23
Fetus	12	25	37
Hispid Cotton Rat			
Male	2	6	8
Female	3	5	8
Fetus	10	7	17
Six-Lined Racerunner			
Male	3	7	10
Female	4	4	8

In an attempt to correlate distribution of the beach mouse with vegetative cover, the second part of the 1973 study was initiated with a total of 83 animals being trapped during an 8-day period, 28 June to 3 July 1973. The majority of animals (63) were found in areas with 5 to 60 percent vegetative cover. Within this range, the greatest number of animals trapped (28) were from an area with 40 to 60 percent cover (Figure 18). A similar habitat preference has been observed along the beaches of the Gulf Coast (Reference 13).

Since it had been shown in laboratory studies (Reference 14) that the liver is the primary site of accumulation of TCDD, it was felt that although no differences in histological examination existed between control and treated specimens an analysis of liver tissue was still warranted. Results of the analysis indicated that composite liver tissues contained from 210 to 540 ppt of TCDD. Apparently TCDD persisted in the environment despite the absence of detectable levels of 2,4,5-T Herbicide. Furthermore, the greatest concentration of TCDD in composite liver samples (540 ppt) was found on Grid 1. This

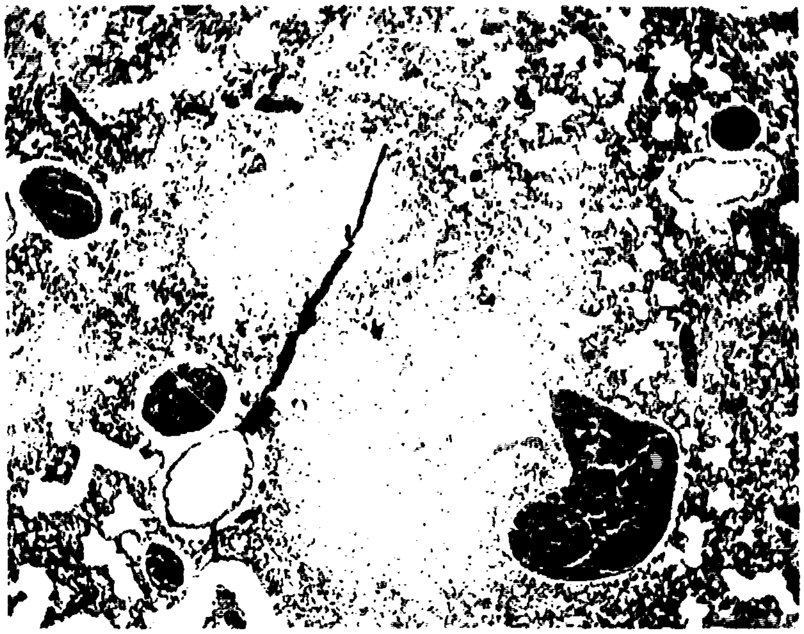

Figure 15. Diffuse Congestion and Edema in the Lung of a Hispid Cotton Rat (*Sigmodon hispidus*) from a Control Area. Hematoxylin and Eosin x 56, AFIP Negative No. 73-8445.

Figure 16. Photomicrograph of Skeletal Muscle of a Beach Mouse (*Peromyscus polionotus*) Taken from the Test Grid. An Encysted Protozoan Parasite (*Sarcocystis* sp) is Seen Within a Muscle Fiber. Hematoxylin and Eosin x 350, AFIP Negative No. 73-8450.

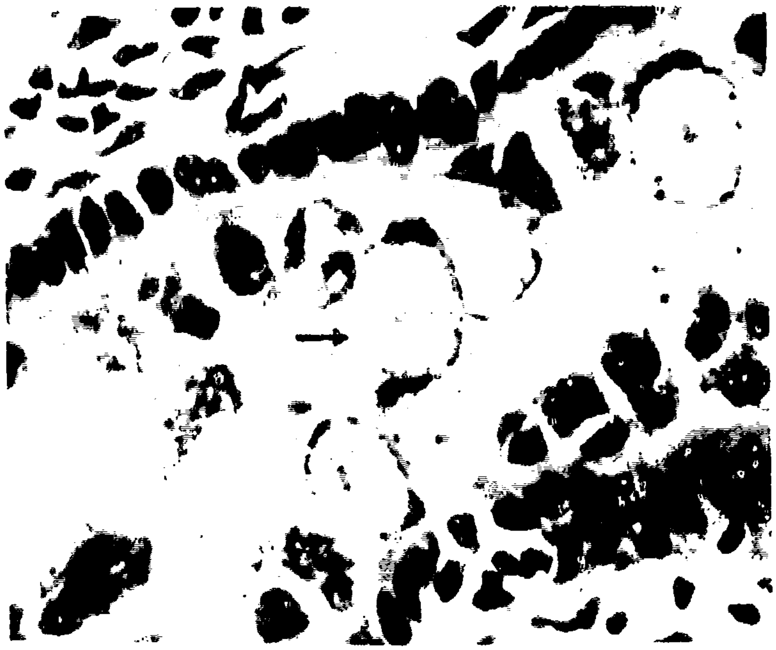

Figure 17. Photomicrograph of a Bile Duct from the Liver of a Six-Lined Racerunner (Cnemidophorus sexlineatus) Taken from the Test Grid. Numerous Coccidian Parasites (Arrow) are Seen Within the Bile Duct Epithelium. Hematoxylin and Eosin x 970. AFIP Negative No. 73-8449.

Figure 18. Typical Habitat for Peromyscus polionotus on Test Area C-52A

area received massive applications of 2,4,5-T Herbicide (87,186 pounds) during the period 1962 through 1964. An analysis of the soil from this site in October 1973, 9 years after the last application, contained 710 ppt of TCDD in the top 6 inches of soil core.

2. CURRENT STUDIES ON ANIMALS

The 1973 data strongly suggested a correlation between liver levels of TCDD and soil levels of TCDD. The current studies were initiated to further explore this relationship. The 1974 studies included (a) an extensive histopathologic examination of beach mice tissues from Grid 1, (b) an investigation of burrow construction and diet of beach mice, (c) an examination of the soil profile for potential zoning of TCDD, (d) further chemical analysis of liver tissue for TCDD presence in male and female beach mice, (e) a laboratory experiment to demonstrate one possible route of TCDD uptake by beach mice, (f) analysis of reptile tissue for TCDD, and (g) collecting tissues for an electron microscope ultrastructure study.

3. MATERIALS AND METHODS

a. Test Animals

The beach mouse (Peromyscus polionotus) is also called a field mouse. It is a small mouse (Figure 19) approximately 120 mm in length, with brown (adult) or dark gray (juvenile) fur on the back, and pale gray to white fur on the ventral region and legs. The species appears to be in the process of splitting into two species with five new subspecies recently described (Reference 15). It may be found in old field habitats and in areas of 5 to 60 percent vegetative cover (Figure 18), but prefers sandy areas. Although the life-span of the beach mouse has been reported to be 5 years in captivity (Reference 16), it is highly unlikely that any of the specimens examined were present on the grid during the final period of spraying.

The racerunner (Cnemidophorus sexlineatus) (Figure 20), the prevalent reptile on the grid, has adapted to a wide range of ecological conditions. It is a fast-moving lizard, found in areas of 10 to 90 percent vegetative coverage. On occasion, these animals may be the principal species in an otherwise uninhabited area. Racerunners from such areas may be used to study the impact on animal life from a defoliated area such as observed at various locations on the test site.

Traps used to capture the mice and reptiles for this study were Havahart® traps. (Havahart Traps, Department 1, P. O. Box 551, Ossining, New York 10562), sizes 0 and 1 for small mammals (Figure 21). These traps were baited with peanut butter and oatmeal. Some traps were randomly placed on the test grid where 20 to 80 percent vegetative coverage was present, while others were placed near openings to mouse burrows. Still other traps were placed in rectangular patterns of five rows of traps, each row located 20 paces apart, and containing five traps per row, at 15-pace intervals. Four

Figure 19. Typical Beach Mouse, Peromyscus polionotus, from Test Area C-52A

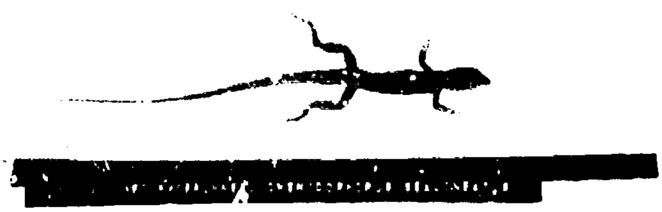

Figure 20. Typical Six-Lined Racerunner, Cnemidophorus sexlineatus, from Test Area C-52A

areas approximately 200 to 1000 yards off the grid were designated as control areas, and were trapped in the same manner as on the test grid. Traps were checked daily and were moved to other locations after 4 days failure to catch an animal. All captured mice and reptiles were taken to the laboratory for histopathologic examination and chemical analysis of the tissues.

All animals were prepared for examination using a cervical dislocation procedure to accomplish humane euthanasia. Euthanatized animals were photographed, weighed, measured, and systematically examined for developmental defects such as cleft palate, cleft lip, polydactyly, and microophthalmia. All internal organs were examined for gross lesions and individually weighed. Representative sections of each tissue were placed in neutral 10 percent buffered formalin and processed for microscopic study by the Veterinary Pathology Division, Armed Forces Institute of Pathology, Washington, D. C. 20305. All remaining tissues from mice captured in the test and control areas were pooled according to sex and maturity, placed in glass jars, frozen, and sent to Interpretive Analytical Services, Dow Chemical, Midland, Michigan, for TCDD analysis.

It should be noted that liver and thymus tissues were also selected for ultrastructural studies. After the liver and thymus were removed and weighed, small blocks of tissue were minced and transferred to containers of the primary fixative, glutaraldhyde buffered with phosphate. Fixation was allowed to continue for 2 hours at 4°C. After fixation, the tissue was rinsed with buffer solution to remove any excess fixative. The small pieces of tissue were post-fixed for 1 hour at 4°C with phosphate-buffered 1 percent osmium tetroxide. The tissue was rinsed again with buffer solution prior to dehydration with a graded series of acetone.

The use of propylene oxide as an intermediate solvent between the dehydrating agent and the epoxy mixture was not required with acetone as it is with ethanol. Therefore, the tissue was transferred directly from 100 percent acetone, after dehydration, to a solution of acetone and the embedding medium and eventually to the embedding medium. The embedding medium of choice was Epon-812®, an excellent preserver of cellular ultrastructures. After the Epon® blocks cured they were set aside for future study and evaluation.

b. Beach Mouse Burrow and Diet Study

To document burrowing habits and help in determining the diet of beach mice it became evident that the burrow itself would have to be examined. Several methods were used to accomplish this task. First, traps were set very close to burrows showing recent mouse activity in hopes of catching as many of the inhabitants as possible (Figure 22). After 4 days of trapping at each selected burrow the next step involved a study of the burrow construction. Two methods were employed to accomplish this task. The first method involved the pouring of a very thin mixture of plaster of paris into the burrow until it was filled. The plaster of paris mixture

Figure 21. A Size 0 Havahart® Trap, Baited, Set, and in Place on Test Area C-52A

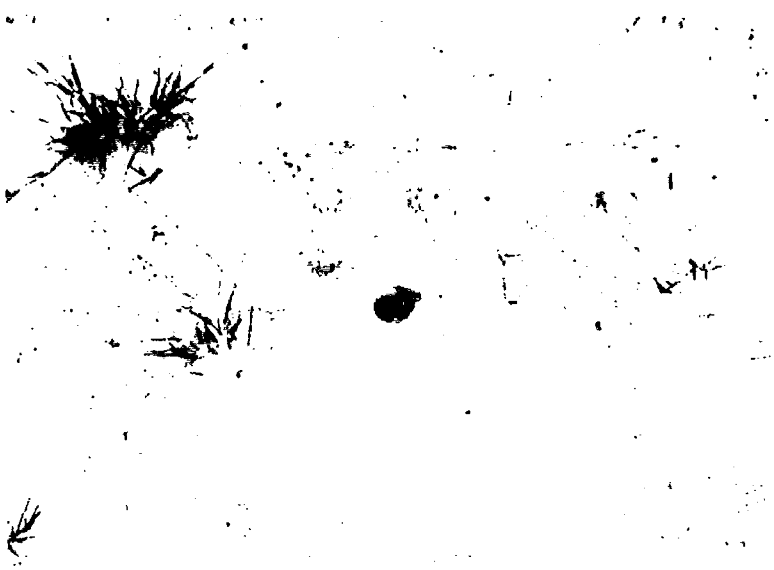

Figure 22. A Representative Beach Mouse Burrow Typical of Those Found on Test Area C-52A

was allowed to dry for 24 hours in the filled burrow. The resulting plaster cast was carefully dug up, revealing the general pattern of construction as shown in Figure 23. The second step used to study the burrow construction involved the careful digging along and in front of the burrow to follow its construction (Figures 24, 25, and 26). As each successive cut was made into the soil, measurements were taken and a sketch of the burrow construction was assembled.

The nest material was carefully removed and placed in individual plastic containers to be taken back to the laboratory for microscopic study to determine plant and seed species present and the presence of any other material that might give a clue to the beach mouse diet.

c. Zoning of TCDD in Soil Profile

Previous analysis of soil samples indicated TCDD was detected only in the top 6 inches of soil (for example, analysis of soil cores to a depth of 3 feet indicated no detectable TCDD in increments below 6 inches). Therefore, selected sites on Grid 1 and the 1-square-mile grid were sampled for TCDD at increments of 0 to 1, 1 to 2, 2 to 4, and 4 to 6 inches.

d. Chemical Analysis of Soil and Tissue for TCDD

The following method for the analysis of TCDD in soils and biological tissues was furnished by Interpretive Analytical Services, Dow Chemical, Midland, Michigan.

Soil Samples:

A 10-gram sample plus 7 milliliters of 1 percent aqueous ammonium chloride was extracted with 100 milliliters of 1:1 acetone: hexane [J. Assoc. Offic. Anal. Chem., 56, 728 (1973)]. The acetone was removed from the hexane by washing with water, then the hexane layer was shaken with 10 milliliters of concentrated sulfuric acid. The hexane layer was evaporated, and the residue was transferred to a 0.4x5-centimeter column of silica gel; the column was eluted with 20 percent benzene in hexane. The residue obtained from evaporation of the eluate was placed on a 0.4x5-centimeter column of activated alumina, and the alumina column was washed with 20 percent CCl_4 in hexane to remove most of the di (p-chlorophenyl) dichloroethylene (DDE) and polychlorinated biphenyls (PCB's). Then the alumina column was eluted with 20 percent methylene chloride in hexane. The eluate was concentrated to a small volume, and aliquots analyzed by gas chromatograph-mass spectrometer, as follows:

An LKB 9000S computerized gas chromatograph-mass spectrometer combination was employed. A 1.83-meter by 2-millimeter glass column, packed with 3 percent Ov-3 silicone on Gas Chrom Z, was used isothermally at 230°C. Detection and measurement was done using the m/e = 320 and m/e = 322 peaks in the mass spectrum $C_{12}H_4O_2Cl_4{}^{35}$ and $C_{12}H_4O_2Cl_3{}^{35}Cl^{37}$ [Environmental Health Perspectives, 5, 15 (Sep 1973)].

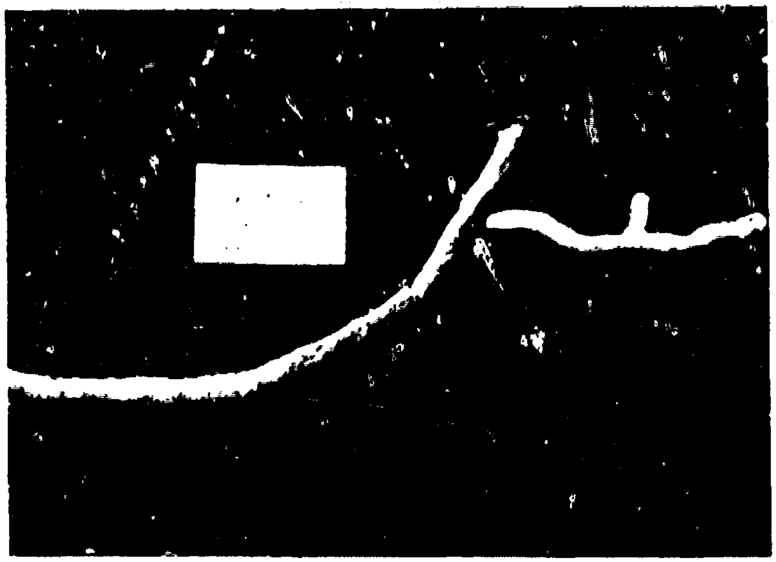

Figure 23. A Common Pattern of Burrow Construction of the Beach Mouse Inhabiting Test Area C-52A. Note the Escape Tunnel Built at a Right Angle to the Main Tunnel but Not Reaching the Surface. The Average Depth of the Tunnel was 21 Inches and Average Length was 55 Inches.

Figure 24. The Initial Removal of Soil From a Beach Mouse Burrow on Test Area C-52A

Figure 25. After the Initial Clearing Away of Soil the Burrow was Examined to Determine its Direction of Construction

Figure 26. When the End of the Burrow was Reached it was Common to Find a Nest Constructed of Plant Materials

Biological Samples:

Ten grams of sample (or the entire sample in cases where 10 grams were not available) was digested with an aqueous ethanol solution of KOH. The resulting solution was extracted with hexane, and the hexane layer then shaken with 10 milliliters of concentrated sulfuric acid. The hexane layer was worked up and analyzed in the same manner from this point as was done for the soil samples [Environmental Health Perspectives, 5, (Sep 1973)].

Results on soil samples are corrected for average 80 percent recovery. Results on biological samples are corrected for average 75 percent recovery. Recoveries were determined by addition of Cl^{37} isotopically enriched TCDD to selected samples and measuring the amount of Cl^{37} TCDD at m/e = 328.

e. TCDD Uptake Experiments in the Laboratory

Because the 1973 data suggested the apparent interrelationship of liver and soil levels of TCDD, a laboratory experiment was designed to simulate a probable source of contact for the beach mouse. When soil studies revealed that 2,4,5-T herbicide and TCDD were confined to the upper 6 inches of soil, it was suggested that the source of contamination to the beach mouse might be related to food source (e.g., seeds). An examination of seeds collected from plants growing on the contaminated soil revealed no TCDD at a detection limit of 1 ppt. However, when it was observed that rodents spend as much as 50 percent of their active hours grooming, another mechanism of contamination was proposed. As the rodents move in and out of their burrows they pass back and forth through the TCDD-laden 6 inches of soil. This soil adheres to their pelts (fur). As a result of the meticulous grooming habits of the beach mouse TCDD could be ingested.

Beach mice captured from the designated control areas were brought into the laboratory and individual animals were placed in separate Isocages (Carworth, Division of Becton, Dickinson & Co., New York City, Rockland County, New York) and maintained on laboratory chow (Ralston Purina Company, General Offices, Checkerboard Square, St. Louis, Missouri). The animals were held until sufficient numbers of mature individuals were on hand to begin the study. When 23 animals were available, they were weighed, sexed, and randomly divided (using a random numbers table to assign numbers and groups) into a control group of 11 animals (four females and seven males) (Figure 27) and a test group of 12 animals (five females and seven males).

The experimental procedure was conducted by dusting each test animal with 100 milligrams of alumina gel containing TCDD at a concentration of 2.50 parts per billion (ppb) using an artist's camel's hair brush (Figure 28) for application. Control animals were dusted with alumina gel alone. Areas dusted included fur on the ventral thoracic and abdominal regions, sides, back, and tail. These areas were observed to receive the most grooming attention from the mouse. The dusting procedure was repeated every third

Figure 27. Control Animals in Iso-Cages Being Held for TCDD Uptake Study

Figure 28. Applying Alumina Gel and TCDD to Ventral Abdominal Region with an Artist's Camel's Hair Brush

day for a total of ten applications during a 28-day period. No attempt was made to quantify the amount of TCDD applied based on weight, but the 100-milligram application per animal resulted in an approximate exposure of 60 milligrams of powder for each application per animal (based on average weight of recovered residue following dusting).

On the twenty-ninth day all animals were euthanatized and necropised. Body and organ weights were recorded, internal organs were examined for gross lesions, and representative sections of each tissue were placed in neutral 10 percent buffered formalin and processed for microscopic study by the Veterinary Pathology Division, Armed Forces Institute of Pathology, Washington, D. C. 20305. It should be noted that liver samples were collected for electron microscope studies prior to preparing the histological sections. The remaining liver tissue and skin were pooled according to sex and test and control, placed in glass jars, frozen, and sent to Interpretive Analysis Services, Dow Chemical, Midland, Michigan, for TCDD analysis.

f. Analysis of Six-Lined Racerunner, Cnemidophorus sexlineatus

The dominant reptilian species observed and collected on the spray equipment testing grids of Test Area C-52A was the six-lined racerunner. This species is extremely adaptable and occupies a variety of niches both on and off the grid. Because of its ability to move rapidly, it is ideally suited to areas of low vegetative cover. Moreover, racerunners were frequently observed at the entrance of beach mouse burrows and on numerous occasions were found trapped in the Havahart® traps. This unexpected trapping presented an abundant source of biological material. The racerunners, apparently by chance, walked into the traps and were caught. With this apparent randomness, there should be no reason to suspect that any one population represents a greater proportion of matures or immatures. No differentiation of maturity was attempted because microscopic examination of the gonads was not performed. However, it is known that males are more aggressive in the field and, therefore, are more likely to be trapped. Thus, a higher proportion of males to females was expected.

As the reptiles were collected they were brought to the laboratory for sexing, weighing, and separation of viscera from trunk. Sexing was accomplished using color (an intense blue throat) to identify males as well as an examination of reproductive organs. The reptiles were cooled to 32°F to slow their movements and then were euthanatized by decapitation. Samples of test and control, male and female, trunk and viscera were pooled and placed in glass jars and frozen. These samples were also analyzed for TCDD. No gross abnormalities were seen and since no abnormalities have been reported in the literature, histopathological examinations were not performed on the racerunners.

4. RESULTS

a. Soil Analysis

Analysis of 6-inch soil cores for TCDD taken at six locations on the 92-acre area (Grid 1) indicated wide fluctuations in TCDD concentrations. The results for the uniformly mixed top 6-inch increments were 10, 25, 70, 70, 110, and 710 parts per trillion (ppt) TCDD. Further analysis of a duplicate core, obtained from the site having 110 ppt TCDD concentration, indicated that TCDD was stratified within the top 6 inches of soil. The analysis for depths of 0 to 1, 1 to 2, 2 to 4, and 4 to 6 inches resulted in detectable levels of 150, 160, 700, and 44 ppt TCDD, respectively.

b. Trapping Data

In the 8 weeks of trapping beach mice during the summer (June-July) of 1973 and 6 weeks during the summer (June-July) of 1974, 106 specimens were collected from either Grid 1 or a portion of a grid immediately north and slightly overlapping Grid 1, and a control site. Since many of the females were pregnant at the time of collection, 67 fetuses were recovered. This brought the total number of beach mice collected and examined over a period of 2 years to 173 (Table 8).

c. Burrow and Diet

From an examination of the burrows it was apparent that no two burrows were identical. However, most were characterized by a small mound of soil on the surface with a 2-inch-diameter tunnel near the center, leading down and away from the surface at a 45-degree angle. A plug of soil was usually found within the first 10 to 12 inches of the tunnel entrance. Eight to 10 inches beyond the plug, the tunnel leveled and continued horizontally for another 18 inches. There it expanded into a spherical chamber with a diameter of about 6 inches in which a nest was usually found. This placed the nest approximately 12 inches below the soil surface. Frequently, beyond the nest, the burrow turned upward and to one side while at the same time narrowing to its original 2-inch diameter. This escape tunnel normally extended to within 2 to 6 inches of the soil surface.

The nests were constructed of dried grasses (stems, blades, and inflorescences). The dominant grasses used for bedding were broomsedge and low panicum. The litter within the nests consisted of caryopsis hulls, leaf fragments, twigs, insect exoskeletons, insect wings, and snake scales. From a detailed examination of the caryopsis hulls, six species comprised the bulk of the vegetative portion of the diet: rough buttonweed, *Diodia teres*; spotted spurge, *Euphorbia maculata*; bitter polygala, *Polygala polygama*; common polypremum, *Polypremum procumens*, and low panicum and switchgrass. Examination of insect parts indicated insects of the Orders Coleoptera (Beetles), Hemiptera (True Bugs), and Homoptera (Cicadas and Leafhoppers, etc). Based upon number of insect parts and seed hulls and upon each of their estimated weights, it was assumed that about 90 percent of the beach mouse diet was seeds and the remaining 10 percent was made up of insects.

TABLE 8. NUMBERS OF BEACH MICE COLLECTED DURING THE 1973 AND 1974 STUDIES OF TEST AREA C-52A

CONTROL	1973	1974	
MALE	5	12	17
FEMALE	5	10	15
FETUSES	12	11	33
		SUBTOTAL =	65
TEST			
MALE	26	17	43
FEMALE	18	13	31
FETUSES	25	9	34
		SUBTOTAL =	108
		TOTAL =	173

The results from the TCDD analysis of the four composite seed samples indicated that TCDD was not detectable in any sample (minimum detection limit of 1 ppt TCDD). The insects were not analyzed for TCDD.

d. Liver and Pelt Analysis

Results of liver and pelt analysis for TCDD are shown in Table 9. Samples of liver, as well as pelts, of mice taken from Grid 1 in which significant soil levels of TCDD were found, exhibited positive evidence of accumulation of TCDD. TCDD was also found in the pooled liver samples of both male and female control animals, although no TCDD was detected on their pelts.

e. Histopathology

A series of histological examinations were performed on the heart, lungs, trachea, salivary glands, thymus, liver, kidneys, stomach, pancreas, adrenals, large and small intestine, spleen, genital organs, bone, bone marrow, skin, and brain. Initially, the tissues were examined on a random basis without the knowledge of whether the mouse was from a control or test area. All

TABLE 9. CONCENTRATION (PARTS PER TRILLION) OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN LIVER AND PELT SAMPLES FROM BEACH MICE, PEROMYSCUS POLIONOTUS COLLECTED FROM CONTROL AND TCDD-EXPOSED FIELD SITES, 1973 AND 1974

TREATMENT	YEAR	SEX	LIVER	PELT
CONTROL	1973	MALE AND FEMALE	20[a]	N.D.[b]
CONTROL	1974	MALE	51	40[a]
		FEMALE	83	40[a]
GRID I	1973	MALE AND FEMALE	540	N.D.[b]
GRID I	1974	MALE	1,300	130
GRID I	1974	FEMALE	960	140

[a] Minimum level of detection.

[b] Not determined.

microscopic changes, including those interpreted as minor or insignificant, were recorded. For example, the following types of lesions were interpreted as not significant or of a common finding when large populations of wild animals are surveyed histologically:

- Variation of nuclear size (poikilotosis) and of cytoplasmic staining in liver tissue from both control and TCDD-exposed animals.

- Subacute, focal myocarditis and sialitis in two separate mice captured from a control area.

- Poikilotosis of acinar cell nuclei in a mucous salivary gland and poikilotosis of nuclei from the adrenal cortices of two separate TCDD-exposed mice.

- Sarcosporidiosis of skeletal muscle in one TCDD-exposed mouse.

Following the recording of all microscopic findings, the tissues were re-examined on a control and test basis. Results of both studies determined that the test and control mice could not be distinguished on an histopathologic basis. Significant lesions were found in only two mice, one from a control area (Figure 29) and one from a test area (Figure 30). Both had a moderately severe, multifocal, necrotizing hepatitis. Sections from the liver of these animals were stained with a variety of stains in attempts to identify an etiologic agent. Neither bacterial nor fungal organisms nor ceroid pigments were demonstrated. The lesions were considered to be virus induced, as they resembled the lesions seen in viral hepatitis of laboratory mice. The gross lesions observed in the kidney of one other beach mouse proved to be severe ectasia of the renal veins (Figures 31, 32, 33, 34, and 35). Microscopically, the vascular dilation was interpreted as being of little functional significance. All other lesions observed in both control and test mice were minor and insignificant and of the type normally observed when a large group of animals are examined at the microscopic level.

Photomicrographs (Figures 36 through 45) are provided to illustrate a random sampling of normal and abnormal tissues examined during this extensive study. AFIP negative numbers are provided for each photomicrograph to assist individuals in obtaining photomicrographs or glass slide specimens of the original tissue so they may perform their own microscopic observations.

Due to the small size of the beach mouse and since both mature and immature specimens were collected, it was most difficult to grossly dissect the thymus gland from salivary glands, fat, and regional lymph tissues. Upon histological examination it was found that the tissue samples contained one or more of the four tissues. Nevertheless, in those specimens that contained thymus tissue, no histological evidence of thymus atrophy was noted in either control or TCDD-exposed animals. Data on the gross weight of thymus tissue were not statistically analyzed because of the uncertainty as to what other tissues might be in the specimen.

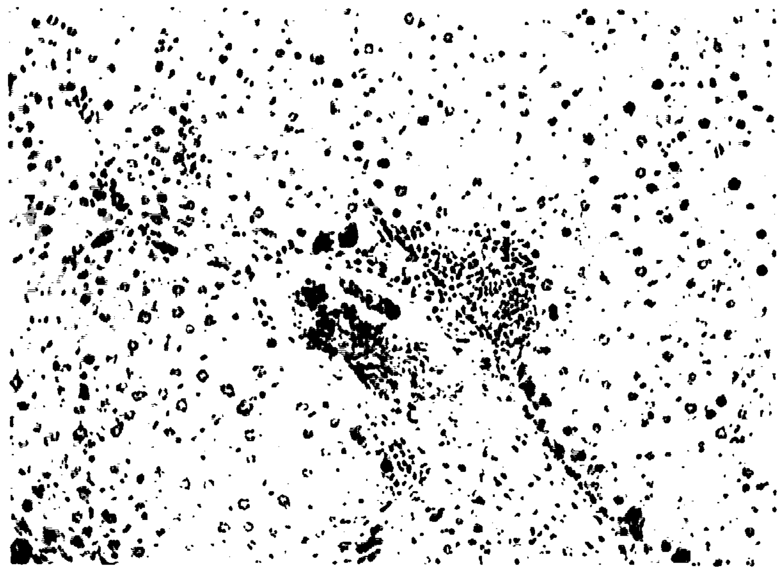

Figure 29. Hepatitis in a Beach Mouse Captured from a Control Area. Inflammatory Cells Can be Seen in the Sinusoids and Periportal Areas. Hematoxylin and Eosin x 130. AFIP Negative No. 74-14883.

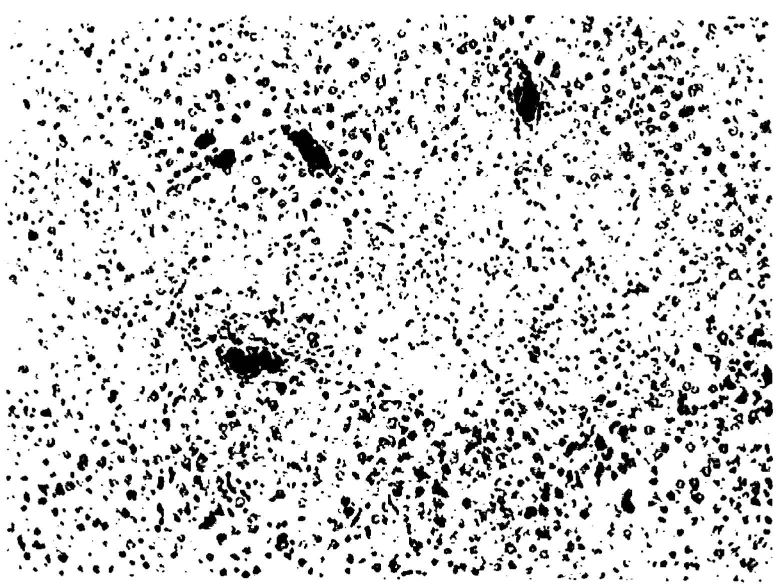

Figure 30. Acute Necrosis and Inflammation in the Liver of a Beach Mouse Captured from the Test Area. Hematoxylin and Eosin x 130. AFIP Negative No. 74-14874.

Figure 31. Gross Illustration Showing Ectasia of Renal Veins on the Left with the Normal Contralateral Kidney on the Right. Beach Mouse was Collected from Test Area. AFIP Negative No. 74-86541.

Figure 32. Close-Up View of Veins Illustrated in Figure 31. AFIP Negative No. 74-86542.

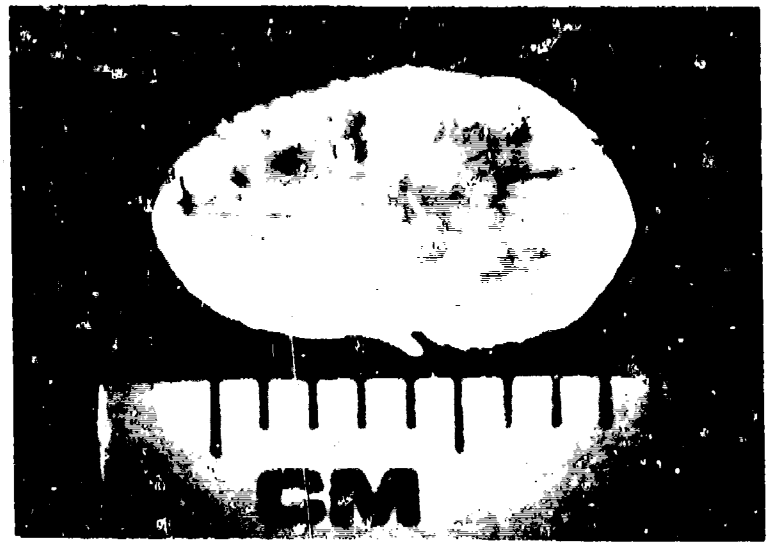

Figure 33. Close-Up View of Normal Contralateral Kidney Illustrated in Figure 31. AFIP Negative No. 74-86543.

Figure 34. Microscopic Appearance of Venous Ectasia in the Kidney of the Beach Mouse Shown in Gross Illustration Figure 31. Hematoxylin and Eosin x 90. AFIP Negative No. 74-14876.

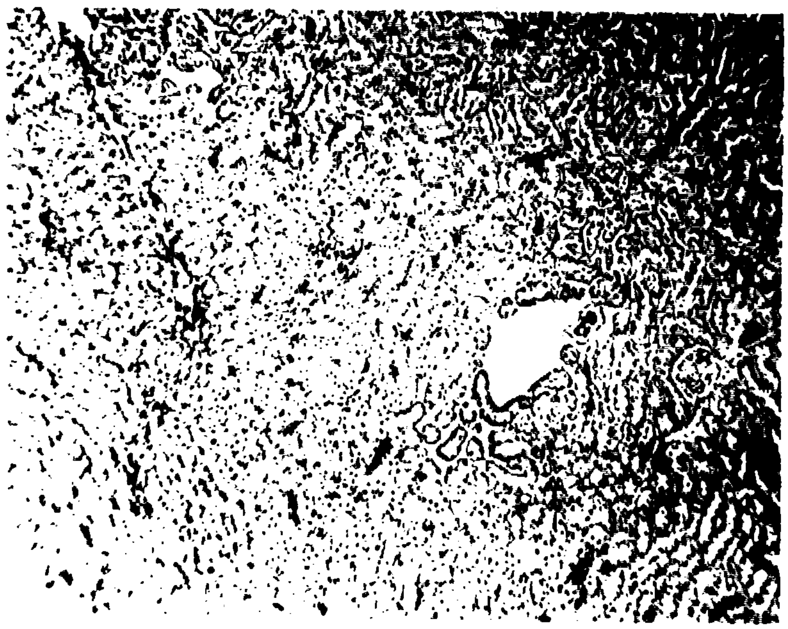

Figure 35. Microscopic Appearance of a Normal Kidney from a Beach Mouse Captured on the Test Area. Compare this Figure with Figure 34. Hematoxylin and Eosin x 90.
AFIP Negative No. 74-14881.

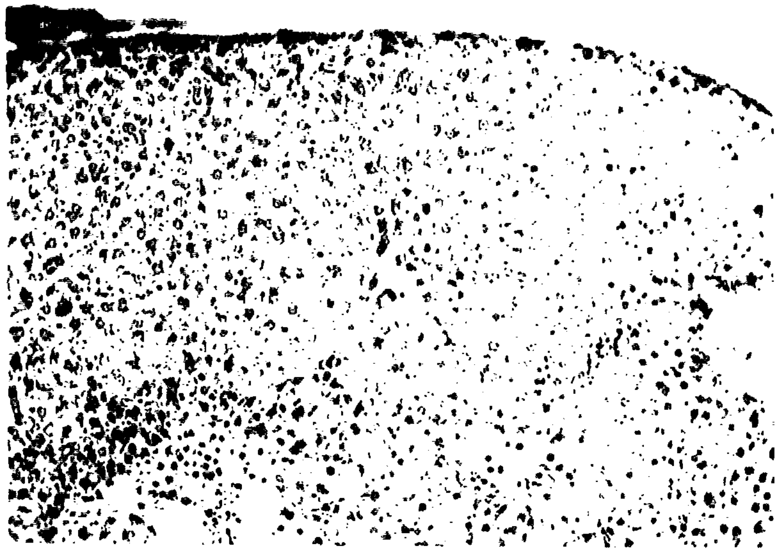

Figure 36. Microscopic Appearance of a Normal Liver from a Beach Mouse Captured on the Test Area. Hematoxylin and Eosin x 130. AFIP Negative No. 74-14875.

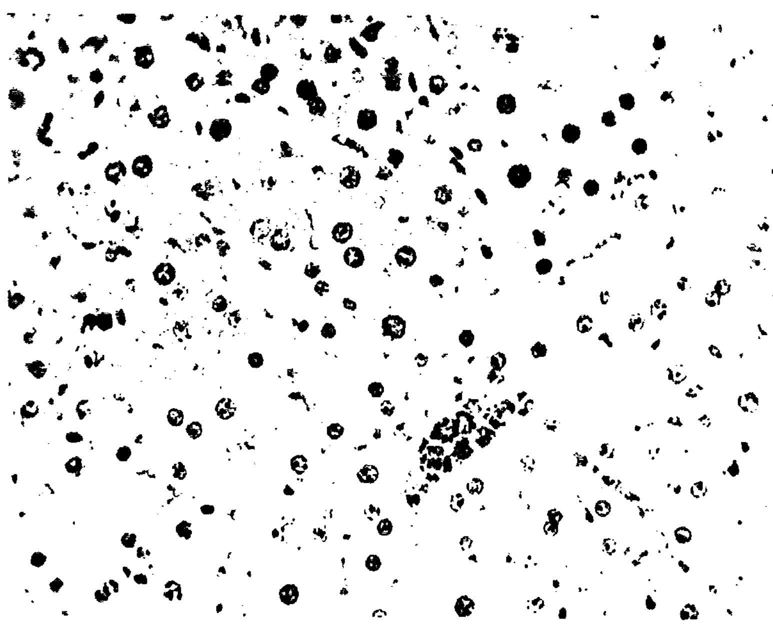

Figure 37. Variation of Nuclear and Cytoplasmic Size and Straining "poikilotosis," Observed in Mice Captured from Both Test and Control Areas. Hematoxylin and Eosin x 180. AFIP Negative No. 74-14882.

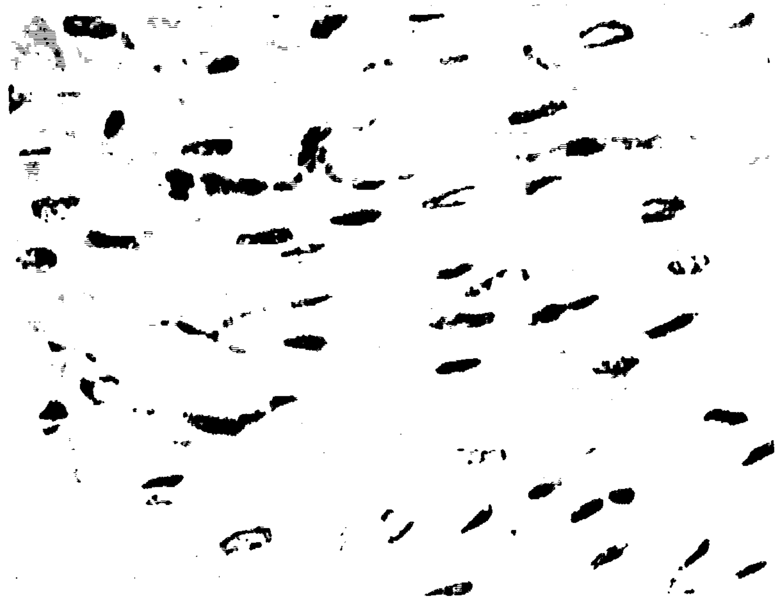

Figure 38. Normal Myocardium of a Beach Mouse Captured in a Control Area. Hematoxylin and Eosin x 675. AFIP Negative No. 74-14886.

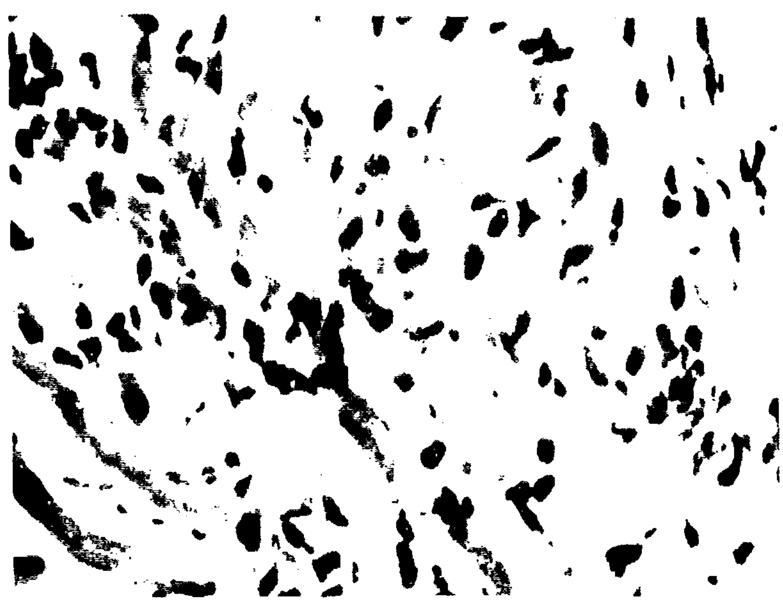

Figure 39. Focal Myocarditis at a Subacute Stage. Same Animal as Figure 30 but Different Area of Heart. Hematoxylin and Eosin x 675. AFIP Negative No. 74-14885.

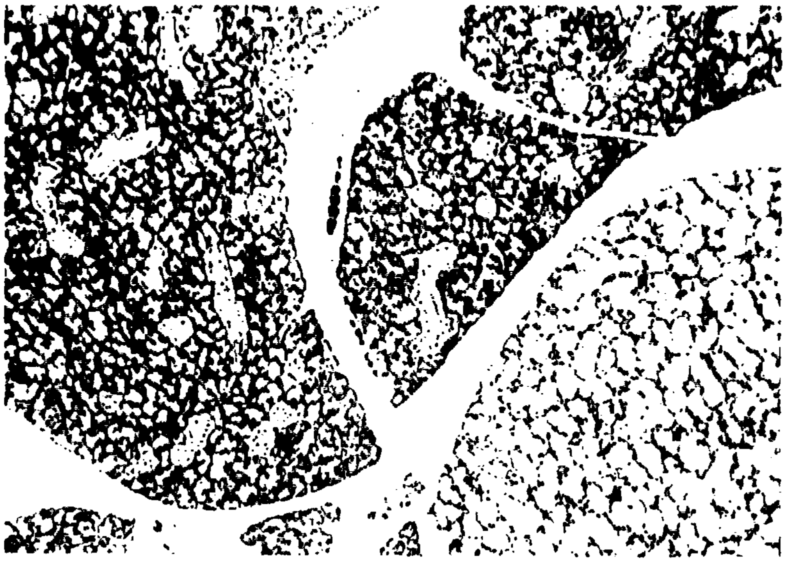

Figure 40. Low Magnification, Microscopic View of a Normal Salivary Gland of a Beach Mouse Captured from the Test Area. Hematoxylin and Eosin x 90. AFIP Negative No. 74-14877.

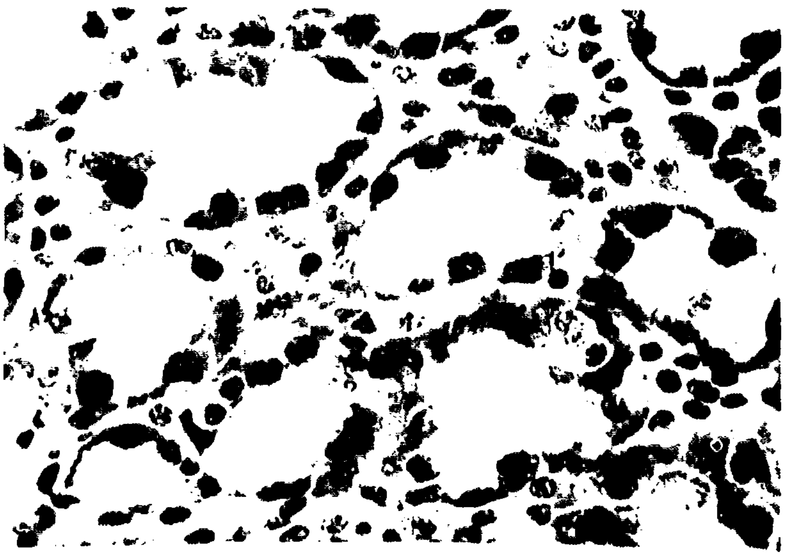

Figure 41. Higher Magnification of a Mucous Salivary Gland of a Beach Mouse Captured from the Test Area. Acinar Cells Show Variation in Nuclear Size. Hematoxylin and Eosin x 675. AFIP Negative No. 74-14873.

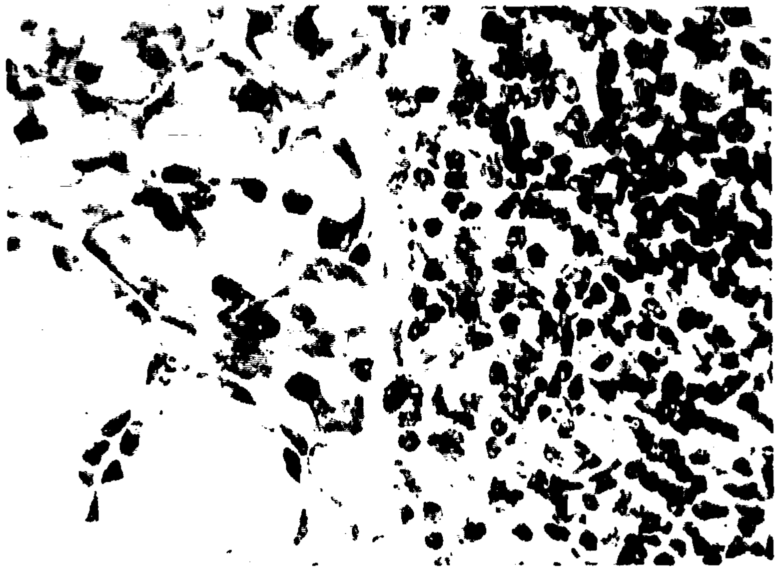

Figure 42. Sialitis in a Control Beach Mouse. Mononuclear Inflammatory Cells within a Mucous Salivary Gland. Hematoxylin and Eosin x 675. AFIP Negative No. 74-14884.

Figure 43. Sarcosparidiosis of the Skeletal Muscle of a Test Area Beach Mouse. Hematoxylin and Eosin x 180. AFIP Negative No. 74-14880.

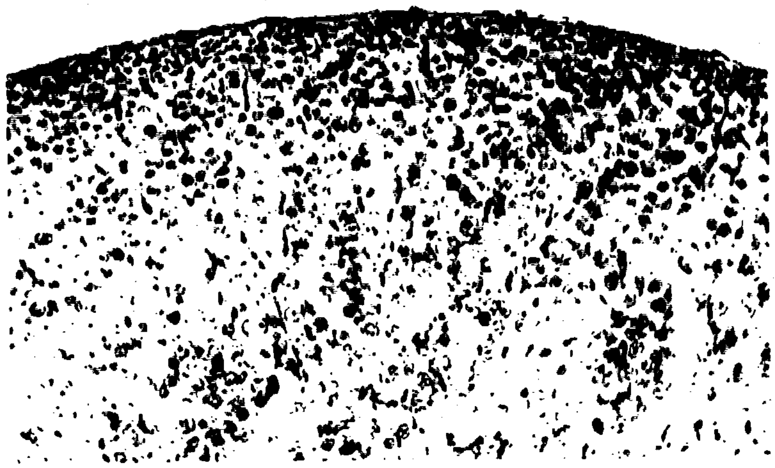

Figure 44. Microscopic Appearance of a Normal Adrenal Gland from a Test Area Beach Mouse. Clear Spaces Represent Artifacts of Preparation. Hematoxylin and Eosin x 180. AFIP Negative No. 74-14879.

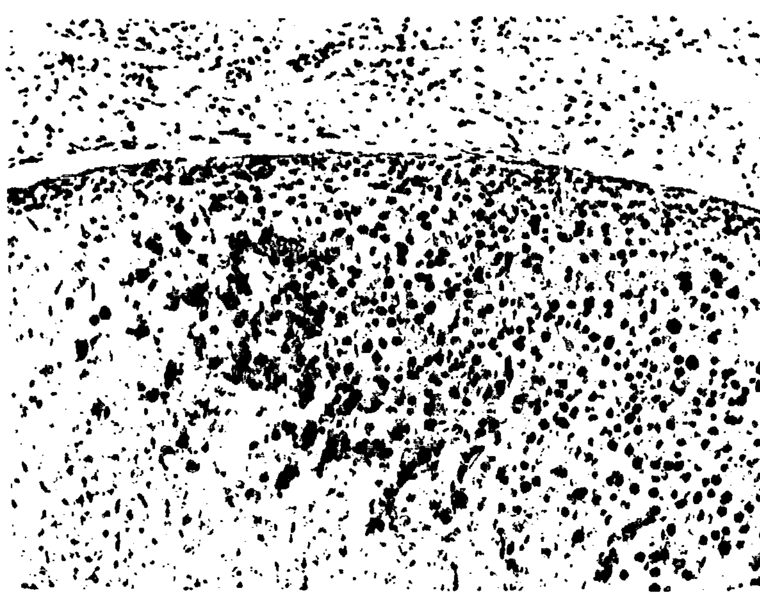

Figure 45. Poikilotosis of the Nuclei of the Adrenal Cortex. This Tissue is from a Beach Mouse Captured on the Test Area. Hematoxylin and Eosin x 180. AFIP Negative No. 74-14878.

f. Statistical Analysis

Tables 10 through 19 present statistical data for physical parameters on beach mice collected from a control site and the TCDD-exposed field site on Test Area C-52A. Pregnant females and sexually immature animals were excluded from statistical comparisons because of the widely varying differences in both body and organ weights. Although microscopic examination for ovarian follicles or sperm production confirmed sexual maturity, animals with total body weights of 10 grams or greater were considered mature and were included in statistical evaluations. Fourteen of 46 females were pregnant. Since their body weights ranged from 11.48 to 18.68 grams, inclusion of these animals in the population used for statistical comparisons would have distorted the data for body and organ weights. Tables 10 and 11 present the raw data while Table 12 presents actual numbers (57) of beach mice used in the statistical analyses. Unfortunately, all females from the 1973 control site were either pregnant or immature.

Table 13 gives the mean total body weight by sex for mature beach mice captured in 1973 and 1974 from control and test sites. A matrix of F-values from the analysis of variance for total body weight for all possible combinations of treatment (location), sex, and year are given in Table 20. Statistically significant differences (95 percent probability level) existed only for sex, or a sex-year interaction. No treatment differences were noted. In general, female beach mice are heavier than male beach mice.

Table 15 presents the mean values for liver weight by sex for mature beach mice captured in 1973 and 1974 from control and test sites. The matrix of F-values from the analysis of variance for all possible combinations of treatment, sex and year are given in Table 16. Statistically significant differences were found for liver weight between sex (e.g., comparing control females collected in 1974, comparison II and III) and with the sex-year interaction (e.g., comparing control 1973 males with control 1974 females). However, the comparison of 1973 or 1974 control males with 1973 or 1974 Grid 1 males (I with IV and II with V, respectively) yielded no significant differences in liver weights. The comparison of liver weights for females collected from a control site in 1974 (III) with females collected in 1974 from the TCDD-exposed site (VII) indicated statistical significance at the 99 percent confidence level. Note from Table 15 that the mean values for liver weight would have suggested the results of the above comparisons.

Table 17 presents mean values for heart, lung, kidney and spleen weights collected in 1974 from beach mice inhabiting control and TCDD-exposed field sites. Statistical analyses of the weights for heart, lung, or kidney indicated no significant differences in the weights of these organs between sex or treatment. However, significant differences were noted for spleen weights between control and test site animals. A matrix of the F-values from the analysis of variance for spleen weights is given in Table 18. Note that the comparison of sex within the same treatment is not significant, e.g., I with II or III with IV. Differences are noted for all treatment comparisons, e.g., I with III, I with IV or II with III, II with IV.

TABLE 10. BODY WEIGHT AND ORGAN WEIGHT DATA FOR *PEROMYSCUS POLIONOTUS* FROM A CONTROL FIELD SITE. (DATA FOR PREGNANT FEMALES AND FOR MICE WITH TOTAL BODY WEIGHTS LESS THAN 10 GRAMS ARE NOT LISTED.)

YEAR COLLECTED	SEX	BODY WEIGHT (GRAMS)	ORGAN WEIGHTS (MILLIGRAMS)				
			HEART	LUNGS	LIVER	SPLEEN	KIDNEYS
1973	M	12.86	70	---[a]	660	---	---
1973	M	11.90	70	---	750	---	---
1973	M	12.30	70	---	880	---	---
1973	M	10.44	100	---	540	---	---
1974	M	14.65	108	119	811	17	226
1974	M	12.62	93	68	778	12	183
1974	F	11.61	77	99	642	26	171
1974	M	12.66	113	108	524	21	199
1974	F	12.55	85	164	688	16	223
1974	M	12.59	96	112	495	14	207
1974	F	10.23	84	88	679	25	170
1974	M	10.44	84	94	580	16	174
1974	M	11.70	130	92	537	16	195
1974	M	12.75	105	102	530	20	174
1974	M	11.72	90	100	726	15	211
1974	M	11.45	100	96	548	20	171
1974	M	11.05	90	87	549	4	203
1974	M	10.55	97	107	643	30	161
1974	F	12.67	121	106	704	20	225

[a]Data not collected

TABLE 11. BODY WEIGHT AND ORGAN WEIGHT DATA FOR PEROMYSCUS POLIONOTUS FROM TCDD-EXPOSED FIELD SITE (TA C-52A). (DATA FOR PREGNANT FEMALES AND FOR MICE WITH TOTAL BODY WEIGHTS LESS THAN 10 GRAMS ARE NOT LISTED.)

YEAR COLLECTED	SEX	BODY WEIGHT (GRAMS)	ORGAN WEIGHTS (MILLIGRAMS)				
			HEART	LUNGS	LIVER	SPLEEN	KIDNEY
1973	M	12.59	100	---[a]	450	---	---
1973	F	14.20	80	---	1150	---	---
1973	M	11.50	30	---	---	---	---
1973	M	11.36	110	---	---	---	---
1973	F	15.43	70	---	1300	---	---
1973	M	13.72	90	---	850	---	---
1973	M	10.70	90	---	940	---	---
1973	M	13.81	100	---	1300	---	---
1973	F	14.59	80	---	1290	---	---
1973	F	16.01	100	---	1450	---	---
1973	M	10.48	70	---	760	---	---
1973	M	12.16	90	---	570	---	---
1973	M	13.50	---	---	---	---	---
1973	F	10.00	80	---	560	---	---
1973	F	10.79	100	---	1140	---	---
1973	M	12.43	100	---	1150	---	---
1973	F	13.93	80	---	1450	---	---
1973	F	11.30	70	---	580	---	---
1973	M	11.28	80	---	800	---	---
1973	M	12.45	80	---	930	---	---

[a]Data not collected

TABLE 11. BODY WEIGHT AND ORGAN WEIGHT DATA FOR PEROMYSCUS POLIONOTUS FROM TCDD-EXPOSED FIELD SITE (TA C-52A) (CONCLUDED). (DATA FOR PREGNANT FEMALES AND FOR MICE WITH TOTAL BODY WEIGHTS LESS THAN 10 GRAMS ARE NOT LISTED.)

YEAR COLLECTED	SEX	BODY WEIGHT (GRAMS)	ORGAN WEIGHTS (MILLIGRAMS)				
			HEART	LUNGS	LIVER	SPLEEN	KIDNEY
1974	M	10.06	73	80	529	14	187
1974	M	13.63	97	112	696	11	196
1974	M	11.49	113	103	824	29	201
1974	M	12.25	997	124	696	16	234
1974	M	11.26	112	92	419	10	179
1974	F	15.57	111	90	926	17	216
1974	F	16.32	108	82	1044	55	241
1974	M	10.05	149	124	436	12	204
1974	F	12.25	114	121	737	11	197
1974	M	11.74	70	85	797	45	191
1974	M	11.09	84	81	635	9	174
1974	M	11.63	82	84	750	35	204
1974	M	10.61	102	151	645	17	174
1974	F	12.05	85	91	734	16	252
1974	M	12.07	92	96	902	28	232
1974	M	11.30	85	89	587	25	171
1974	M	12.21	75	80	847	58	173
1974	M	11.46	84	98	544	14	189

TABLE 12. NUMBER OF *PEROMYSCUS POLIONOTUS* USED IN STATISTICAL COMPARISONS OF POPULATIONS COLLECTED FROM CONTROL SITE AND TCDD-EXPOSED FIELD SITE (GRID 1, TA C-52A). PREGNANT FEMALES AND IMMATURES EXCLUDED.

LOCATION	SEX	1973	1974
CONTROL	MALE	4	11
	FEMALE	0[a]	4
TREATMENT	MALE	12	14
	FEMALE	8	4

[a]All females were either pregnant or immature

TABLE 13. MEAN VALUES FOR TOTAL BODY WEIGHT (GRAMS) OF *PEROMYSCUS POLIONOTUS* COLLECTED FROM CONTROL SITE AND TCDD-EXPOSED FIELD SITE (GRID 1, TA C-52A). PREGNANT FEMALES AND IMMATURES EXCLUDED.

LOCATION	SEX	1973	1974
CONTROL	MALE	11.88 $\pm$ 1.03	12.02 $\pm$ 1.21
	FEMALE	---[a]	11.77 $\pm$ 1.13
TREATMENT	MALE	12.17 $\pm$ 1.13	11.49 $\pm$ 0.93
	FEMALE	13.28 $\pm$ 2.27	14.05 $\pm$ 2.21

[a]All females were either pregnant or immature

TABLE 14. MATRIX OF F-VALUES FROM ANALYSIS OF VARIANCE OF TOTAL BODY WEIGHT FOR *PEROMYSCUS POLIONOTUS* COLLECTED FROM CONTROL SITE AND TCDD-EXPOSED FIELD SITE (GRID 1, TA C-52A).

MATRIX NUMBER	LOCATION	SEX	YEAR	MATRIX NUMBER						
				I	II	III	IV	V	VI	VII
I	CONTROL	MALE	1973	---	F = 1.37 ns[a]	F = 1.19 ns	F = 1.20 ns	F = 1.24 ns	F = 4.80 P .10	F = 4.58 ns
II	CONTROL	MALE	1974		---	F = 1.15 ns	F = 1.14 ns	F = 1.70 ns	F = 3.50 P .05	F = 3.34 P .10
III	CONTROL	FEMALE	1974			---	F = 1.01 ns	F = 1.47 ns	F = 4.04 ns	F = 3.85 ns
IV	GRID I	MALE	1973				---	F = 1.48 ns	F = 4.01 P .025	F = 3.82 P .05
V	GRID I	MALE	1974					---	F = 5.94 P .005	F = 5.67 P .025
VI	GRID I	FEMALE	1973						---	F = 1.05 ns
VII	GRID I	FEMALE	1974							---

[a]Not significant at a probability of less than 0.10 (i.e., 90 percent confidence level)

TABLE 15. MEAN VALUES FOR LIVER WEIGHT (MILLIGRAMS) OF *PEROMYSCUS POLIONOTUS* COLLECTED FROM CONTROL SITE AND TCDD-EXPOSED FIELD SITE (GRID 1, TA C-52A). PREGNANT FEMALES AND IMMATURES EXCLUDED.

LOCATION	SEX	1973	1974
CONTROL	MALE	707.50 ± 143.61	611.00 ± 111.34
	FEMALE	--- [a]	678.25 ± 26.29
TREATMENT	MALE	861.11 ± 263.36	664.79 ± 150.54
	FEMALE	1115.00 ± 355.65	860.25 ± 151.90

[a]All females were either pregnant or immature

TABLE 16. MATRIX OF F-VALUES FROM ANALYSIS OF VARIANCE OF LIVER WEIGHT FOR *PEROMYSCUS POLIONOTUS* COLLECTED FROM CONTROL SITE AND TCDD-EXPOSED FIELD SITE (GRID 1, TA C-52A)

MATRIX NUMBER	LOCATION	SEX	YEAR	MATRIX NUMBER I	II	III	IV	V	VI	VII
I	CONTROL	MALE	1973	---	F = 1.66 ns[a]	F = 29.85 P .01	F = 3.36 ns	F = 1.10 ns	F = 6.13 P .10	F = 1.12 ns
II	CONTROL	MALE	1974		---	F = 17.94 P .025	F = 5.59 P .01	F = 1.83 ns	F = 10.20 P .005	F = 1.86 ns
III	CONTROL	FEMALE	1974			---	F = 100.39 P .005	F = 32.80 P .01	F = 183.07 P .005	F = 33.39 P .01
IV	GRID I	MALE	1973				---	F = 3.06 P .05	F = 1.82 ns	F = 3.01 ns
V	GRID I	MALE	1974					---	F = 5.58 .005	F = 1.02 ns
VI	GRID I	FEMALE	1973						---	F = 5.48 P .10
VII	GRID I	FEMALE	1974							---

[a]Not significant at a probability less than 0.10 (i.e., 90 percent confidence level)

TABLE 17. MEAN VALUES FOR HEART, LUNG, KIDNEY AND SPLEEN WEIGHTS (MILLIGRAMS) FOR *PEROMYSCUS POLIONOTUS* COLLECTED IN 1974 FROM CONTROL SITE AND TCDD-EXPOSED FIELD SITE. PREGNANT FEMALES AND IMMATURES EXCLUDED.

LOCATION	SEX	ORGAN			
		HEART	LUNG	KIDNEY	SPLEEN
CONTROL	MALE	100.55 ± 12.98	98.64 ± 13.82	191.27 ± 20.12	16.82 ± 6.42
CONTROL	FEMALE	91.75 ± 19.82	114.25 ± 33.98	197.25 ± 30.90	21.75 ± 4.65
GRID I	MALE	93.93 ± 20.75	99.93 ± 21.04	193.50 ± 20.33	23.07 ± 14.66
GRID I	FEMALE	104.50 ± 13.23	96.00 ± 17.15	226.50 ± 24.77	24.75 ± 20.34

TABLE 18. MATRIX OF F-VALUES FROM ANALYSIS OF VARIANCE OF SPLEEN WEIGHT FOR PEROMYSCUS POLIONOTUS COLLECTED IN 1974 FROM CONTROL SITE AND TCDD-EXPOSED FIELD SITE (GRID 1, TA C-52A)

MATRIX NUMBER	LOCATION	SEX	MATRIX NUMBER I	II	III	IV
I	CONTROL	MALE	---	F = 1.91 ns[a]	F = 5.22 P .01	F = 10.05 P .005
II	CONTROL	FEMALE		---	F = 9.96 P .05	F = 19.16 P .025
III	GRID I	MALE			---	F = 1.92 ns
IV	GRID I	FEMALE				---

[a]Not significant at a probability of less than 0.10 (i.e., 90 percent confidence level).

g. TCDD Laboratory Uptake Experiments

The analysis of liver tissue and pelts from beach mice dusted 10 times with alumina gel plus 0 or 2.5 ppb TCDD during a period of 28 days are shown in Table 19. Control animals used in this study were true controls in the sense that no TCDD was detected in the livers or pelts at a minimum detection limit of 8 or 10 ppt, respectively. Those animals receiving the alumina gel plus TCDD had detectable levels on their pelts (45 and 89 ppt for male and female, respectively). However, separate analysis by sex for TCDD in the liver tissue was not possible because of the small amounts of tissue available. Nevertheless, the composite sample contained 125 ppt TCDD.

Body and organ weights for the 10 control and 12 TCDD-exposed beach mice are given in Table 20. Notice that gonad data (weight of ovaries) were not available for females because of the difficulty of separating ovarian tissue from surrounding structures in animals of such small body size.

All remaining organ data were collected at post-mortem examination. Body weights for all 22 animals were obtained prior to the initiation of dusting. These initial values are compared with final body weights in Table 21. Ignoring sex of animals, the data indicated that the control animals exhibited a slight gain in weight during the 28-day study (+0.17 gram) while the test group showed a slight decline (-0.45 gram). The short term effects of ingested TCDD on change in weight as evaluated statistically would lead to an estimated loss of about 0.62 gram in the adult mouse. However, this change is not significant at the 0.05 level; therefore, a null hypothesis of no effect due to TCDD ingestion can be accepted.

Statistical analysis of mean final body weight of beach mice dusted with alumina gel plus or minus TCDD was also performed. The mean data for number, sex and final weight for the animals are presented in Table 22. A matrix of F-values from analysis of variance of final total body weights is given in Table 23. Note that statistically significant differences (99.5 percent confidence level) existed for those comparisons with the control males. Previous data (Table 13) confirmed differences in mean body weights between males and females. Therefore, comparison of I with III (male control with test male) suggests that ingestion of TCDD by the male beach mouse (in the laboratory) has a significant effect on body weight. However, the direction of change is not a weight loss but rather a weight gain.

Mean liver weights for beach mice dusted with an alumina gel plus or minus TCDD are shown in Table 24. A matrix of F-values from the analysis of variance of the liver weights is shown in Table 25. As with body weight data, statistical significance occurred only with comparisons involving control males. The between sex comparison (control male with control female) and the within sex comparison (control male with test male) were both significant at the 95 percent confidence level.

TABLE 19. CONCENTRATION (PARTS PER TRILLION) OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN LIVER AND PELT SAMPLES FROM BEACH MICE, *PEROMYSCUS POLIONOTUS*, DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL) OR 2.5 PARTS PER BILLION TCDD (TEST)

TREATMENT	SEX	LIVER	PELT
ALUMINA GEL	MALE	8[a]	10[a]
	FEMALE	8[a]	10[a]
ALUMINA GEL + TCDD	MALE	125[b]	45
	FEMALE	125[b]	89

[a] Minimum level of detection

[b] Male and female livers composited for analysis

TABLE 20. BODY AND ORGAN WEIGHTS FOR PEROMYSCUS POLIONOTUS DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL) OR ALUMINA GEL CONTAINING 2.5 PARTS PER BILLION TCDD (TEST GROUP)

TREAT-MENT	SEX	BODY WEIGHT (GRAMS)	ORGAN WEIGHTS (MILLIGRAMS)							
			HEART	LUNG	LIVER	SPLEEN	KIDNEY	GONADS	THYMUS	ADRENALS
C[a]	F	17.55	156	112	951	26	258	---[b]	15	41
C	F	16.98	112	106	980	24	255	---	54	49
C	F	11.43	92	80	606	14	201	---	19	46
C	M	12.60	115	95	577	10	199	38	19	26
C	F	14.23	132	95	825	20	230	---	11	28
C	M	12.72	75	95	610	10	186	93	18	22
C	M	14.38	81	125	686	13	207	127	12	26
C	M	13.10	130	79	645	20	197	96	12	30
C	M	13.26	100	101	698	19	118	100	18	43
C	M	12.97	158	118	718	14	190	87	18	27
T	F	12.07	98	84	714	17	195	---	11	30
T	M	15.72	144	107	953	37	226	106	33	25
T	M	12.77	122	90	542	20	189	93	20	32
T	M	18.02	156	123	790	25	225	109	21	42
T	M	13.65	105	88	805	19	246	105	15	34
T	M	13.20	119	92	713	24	202	99	13	39
T	M	15.57	127	90	723	33	214	83	27	59
T	F	11.78	117	80	593	17	196	---	14	20
T	F	12.61	101	112	751	14	219	---	15	28
T	F	14.99	126	113	912	14	279	---	10	35
T	F	13.77	123	88	832	9	243	---	13	31
T	M	14.12	116	109	779	22	226	118	11	27

[a]C = Control Group, T = Test Group
[b]Data not collected

TABLE 21. INITIAL AND FINAL BODY WEIGHTS FOR *PEROMYSCUS POLIONOTUS* DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL GROUP) OR ALUMINA GEL CONTAINING 2.5 PARTS PER BILLION OF TCDD (TEST GROUP)

CONTROL GROUP WEIGHTS (GRAMS)			TEST GROUP WEIGHTS (GRAMS)		
INITIAL	FINAL	DIFFERENCE	INITIAL	FINAL	DIFFERENCE
17.06	17.55[a]	+ 0.44	12.69	12.07	- 0.62
13.50	16.80	+ 3.30	16.10	15.72	- 0.38
11.00	11.43	+ 0.43	13.12	12.77	- 0.35
13.40	12.60	- 0.80	17.15	18.02	+ 0.87
15.25	14.23	- 1.02	13.71	13.65	- 0.06
12.50	12.72	+ 0.22	14.48	13.20	- 1.28
14.01	14.38	+ 0.37	14.90	15.57	+ 0.67
13.12	13.10	- 0.02	12.36	11.78	- 0.58
14.10	13.26	- 0.84	14.03	12.61	- 1.42
13.40	12.97	- 0.43	16.00	14.94	- 1.01
			13.90	13.77	- 0.13
			15.25	14.12	- 1.13

[a]Data on sex of animals are shown in Table 20

TABLE 22. NUMBER, SEX, AND MEAN FINAL BODY WEIGHT (GRAMS) OF *PEROMYSCUS POLIONOTUS* DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL GROUP) OR ALUMINA GEL CONTAINING 2.5 PARTS PER BILLION TCDD (TEST GROUP)

TREATMENT	SEX	NUMBER	MEAN FINAL BODY WEIGHT (GRAMS)
ALUMINA GEL	MALE	6	13.00 ± 0.30
	FEMALE	4	15.00 ± 2.77
ALUMINA GEL + TCDD	MALE	7	14.82 ± 1.99
	FEMALE	5	13.22 ± 1.27

TABLE 23. MATRIX OF F-VALUES FROM ANALYSIS OF VARIANCE OF FINAL TOTAL BODY WEIGHTS OF *PEROMYSCUS POLIONOTUS* DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL GROUP) OR ALUMINA GEL CONTAINING 2.5 PARTS PER BILLION TCDD (TEST GROUP)

MATRIX NUMBER	TREATMENT	SEX	MATRIX NUMBER I	II	III	IV
I	CONTROL	MALE	---	F = 83.42 P .005	F = 42.95 P .005	F = 17.38 P .005
II	CONTROL	FEMALE		---	F = 1.94 ns[a]	F = 4.80 ns
III	TEST	MALE			---	F = 2.47 ns
IV	TEST	FEMALE				---

[a]Not significant at a probability of less than 0.10 (i.e., 90 percent confidence level)

TABLE 24. MEAN LIVER WEIGHT (GRAMS) OF *PEROMYSCUS POLIONOTUS* DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL GROUP) OR ALUMINA GEL CONTAINING 2.5 PARTS PER BILLION TCDD (TEST GROUP)

TREATMENT	SEX	MEAN LIVER WEIGHT (GRAMS)
ALUMINA GEL	MALE	655.67 ± 54.75
	FEMALE	840.50 ± 170.20
ALUMINA GEL + TCDD	MALE	754.33 ± 134.97
	FEMALE	763.50 ± 108.32

TABLE 25. MATRIX OF F-VALUES FROM ANALYSIS OF VARIANCE OF LIVER WEIGHT FOR *PEROMYSCUS POLIONOTUS* DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL GROUP) OR ALUMINA GEL CONTAINING 2.5 PARTS PER BILLION TCDD (TEST GROUP)

MATRIX NUMBER	TREATMENT	SEX	MATRIX NUMBER I	MATRIX NUMBER II	MATRIX NUMBER III	MATRIX NUMBER IV
I	CONTROL	MALE	---	F = 9.67 P .05	F = 6.08 P .05	F = 3.91 ns
II	CONTROL	FEMALE		---	F = 1.59 ns[a]	F = 2.47 ns
III	TEST	MALE			---	F = 1.55 ns
IV	TEST	FEMALE				---

[a]Not significant at a probability of less than 0.10 (i.e., 90 percent confidence level)

Mean values for heart, lung, spleen, kidney, and adrenal weights for animals in the laboratory dusting study are given in Table 26. The only significant comparison involved heart weights, although in the field study spleen weights were significant and heart weight was not (Tables 17 and 18). A matrix of F-values from the analysis of variance of heart weight is given in Table 27. Note that all significant comparisons involve a sex interaction, e.g., I with IV, or II with III.

h. Analysis of Six-Lined Racerunner, <u>Chemidophorus sexlineatus</u>

Chemical analysis for TCDD in body parts of the six-lined racerunner are given in Table 28. Significant levels of TCDD were found in both the visceral mass (all the internal soft tissue including stomach content) and in the trunk (body with limbs and head removed). Gross post mortem examinations were performed on 19 racerunners collected from either a control site or from Grid 1. No evidence of abnormality was detected in any of the specimens.

A statistical analysis was performed on the total body weights, Table 29, obtained at necropsy, for male and female racerunners collected in 1973 and 1974 from test and control areas. Hypotheses concerning no effect due to year of collection, no differences attributable to sex, and no effect due to treatment were tested. The analysis of variance indicated that no significant effects with TCDD were detected relative to variations in total body weights between control specimens and those collected on a site heavily contaminated.

5. DISCUSSION

a. Soil Analysis

The application of massive quantities of 2,4,5-T herbicide to Test Area C-52A has created a unique field site in which to assess not only the ecological impact of the herbicide 2,4,5-T but also the toxic contaminant TCDD. The method of application, i.e., by aerial dissemination, resulted in unequal distribution of herbicide. Three major flight paths intersected the 92-acre instrumented grid. If a soil sample were obtained from an area outside one of the flight paths or if it were obtained near the intersection of all three flight paths, then the residue levels would be expected to vary significantly. The six soil samples previously described were from areas thought to be flight paths, intersection of flight paths, or areas between flight paths. Levels of TCDD found in these samples confirmed the hypotheses that TCDD concentration were greatest within the flight paths.

The data suggest that TCDD may persist for long periods of time in the environment. However, caution must be exercised when making such a statement. These data are shown in Table 30 and are placed in perspective with hypothetical concentrations of TCDD which might occur with currently produced 2,4,5-T herbicide formulations containing 0.1 part per million (ppm) TCDD and

TABLE 26. MEAN VALUES FOR HEART, LUNG, SPLEEN, KIDNEY, AND ADRENAL WEIGHTS (MILLIGRAMS) OF *PEROMYSCUS POLIONOTUS* DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL GROUP) OR ALUMINA GEL CONTAINING 2.5 PARTS PER BILLION TCDD (TEST GROUP)

TREATMENT	SEX	HEART	LUNG	SPLEEN	KIDNEY	ADRENAL
ALUMINA GEL	MALE	109.83 ± 31.29	102.17 ± 16.81	14.33 ± 4.32	182.83 ± 32.59	29.00 ± 7.32
ALUMINA GEL	FEMALE	123.00 ± 27.40	98.25 ± 14.06	21.00 ± 5.29	236.00 ± 26.50	41.00 ± 9.27
ALUMINA GEL + TCDD	MALE	128.83 ± 18.35	98.33 ± 13.92	26.33 ± 7.20	217.00 ± 20.02	38.50 ± 11.64
ALUMINA GEL + TCDD	FEMALE	113.50 ± 11.50	97.67 ± 15.24	15.50 ± 4.32	226.33 ± 31.65	28.50 ± 5.01

TABLE 27. MATRIX OF F-VALUES FROM ANALYSIS OF VARIANCE OF HEART WEIGHT FOR *PEROMYSCUS POLIONOTUS* DUSTED WITH ALUMINA GEL CONTAINING NO TCDD (CONTROL GROUP) OR ALUMINA GEL CONTAINING 2.5 PARTS PER BILLION TCDD (TEST GROUP)

MATRIX NUMBER	TREATMENT	SEX	MATRIX NUMBER			
			I	II	III	IV
I	CONTROL	MALE	---	$F = 1.30$ ns[a]	$F = 2.91$ ns	$F = 7.40$ P .05
II	CONTROL	FEMALE		---	$F = 2.23$ P .05	$F = 5.67$ ns
III	TEST	MALE			---	$F = 2.54$ ns
IV	TEST	FEMALE				---

[a]Not significant at a probability of less than 0.10 (i.e., 90 percent confidence level)

TABLE 28. CONCENTRATION (PARTS PER TRILLION) OF 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN COMPOSITE SAMPLES OF VISCERA OR TRUNK FROM SIX-LINED RACERUNNERS, *CNEMIDOPHORUS SEXLINEATUS* COLLECTED FROM CONTROL AND TCDD-EXPOSED FIELD SITES

LOCATION	VISCERA	TRUNK
CONTROL SITE	50[a]	40[a]
TEST SITE	360	370

[a]Minimum detection limit

TABLE 29. DATA FROM SPECIMENS OF CNEMIDOPHORUS SEXLINEATUS (SIX-LINED RACERUNNER) CAPTURED ON TEST AREA C-52A AND FROM A CONTROL SITE

TREATMENT[a]	YEAR	SEX	BODY WEIGHT (GRAMS)	TREATMENT	YEAR	SEX	BODY WEIGHT (GRAMS)
C[a]	1973	M	6.23	T	1973	M	8.83
C	1973	F	6.96	T	1973	F	8.71
C	1973	F	7.90	T	1973	M	4.76
C	1974	M	4.40	T	1973	F	4.69
C	1974	M	4.61	T	1973	M	4.14
C	1974	M	6.20	T	1974	M	7.00
C	1974	M	8.80	T	1974	F	4.40
C	1974	M	6.40	T	1974	M	7.20
C	1974	M	7.70	T	1974	M	7.12
C	1974	F	7.50	T	1974	F	4.97
C	1974	M	8.48	T	1974	F	5.61
C	1974	M	7.07	T	1974	M	8.17
C	1974	M	7.95	T	1974	M	6.55
C	1974	M	8.68	T	1974	M	5.62
C	1974	M	8.32	T	1974	M	6.14
C	1974	M	5.70	T	1974	M	6.88
C	1974	F	7.43	T	1974	M	3.98
C	1974	M	7.16	T	1974	M	6.30
T	1973	F	6.34	T	1974	M	4.38
T	1973	M	6.07	T	1974	M	4.98
T	1973	F	6.68	T	1974	M	7.51
T	1973	M	4.15	T	1974	M	5.42
T	1973	M	8.43	T	1974	F	8.14
T	1973	M	8.95	T	1974	M	7.59
T	1973	M	8.51				

[a]C = Control Site, T = Test Area

TABLE 30. COMPARISON OF 2,4,5-T/TCDD APPLICATION RATES TO RANGELANDS (NORMAL USE) VERSUS RATES APPLIED TO GRID 1, TEST AREA C-52A, EGLIN AIR FORCE BASE, FLORIDA (MILITARY AERIAL SPRAY EQUIPMENT TEST PROGRAM)

SUBJECT	NORMAL RANGELAND USE	GRID I APPLICATIONS (1962-1964)
Pounds 2,4,5-T Active Ingredient Per Acre	2	947
Total For 92 Acre Area	184	87,186
TCDD Concentration of 2,4,5-T Formulation	<0.1 ppm (Current Production Standards)	<0.1 - 47 ppm[a]
Concentration of TCDD in Soil Profile (parts per trillion):		
0-1 inch	0.8 ppt[b]	150 ppt[c]
1-2 inches	Not Detectable	160 ppt
2-4 inches	Not Detectable	700 ppt
4-6 inches	Not Detectable	44 ppt
Below	Not Detectable	Not Detectable

[a]Range of TCDD Contamination in Herbicide Stocks Returned from Southeast Asia in 1971 and stored on Johnston Island, Pacific Ocean. (In Disposition of Orange herbicide by Incineration, November 1974, Department of the Air Force Final Environmental Statement.)

[b]Assuming no TCDD degradation and the application of 2,4,5-T to bare soil. If 2 pounds 2,4,5-T, containing 0.1 ppm TCDD, are applied and uniformly mixed into top 1 inch of an acre of soil, then $2 \times 0.1 \times 10^{-6}$ pound TCDD per acre in 1 inch of soil weighing 3×10^{6} pound per acre-foot or about 0.25×10^{6} pound per inch per acre equals $0.2 \times 10^{-6}/0.25 \times 10^{6}$ or 0.8×10^{-12}.

[c]Soil profile samples collected in 1974.

applied at normal rangeland or reforestation rates for brush control. As noted in Table 30, it was probable that Grid 1 received highly contaminated herbicide. The herbicide was most likely produced in the 1950s or early 1960s and thus was subjected to preparation treatment different from those controlled procedures subsequently used. A conservative estimate for TCDD contamination may be 8 ppm in the formulation. Using the 8 ppm figure for 4600 pounds of butyl esters of 2,4-D and 2,4,5-T applied per acre (equivalent to 947 pounds of 2,4,5-T acid) in the years from 1962 through 1964, the amount of TCDD applied was 0.0368 pound per acre. This is 12,267 ppt TCDD in the top 6 inches of soil(2). At the least, this has declined to 710 ppt in about 8 years. This is a loss of about 95 percent. Thus, the apparent high residue is probably due to the massive quantities applied rather than to the resistance of TCDD to biological and/or physical degradation.

b. Liver and Pelt Analysis

The presence of TCDD in the liver samples of both male and female mice collected from the control site in 1974 may have been due to high levels in one or more specimens in the pool of samples. Mice from the test area could have migrated to the periphery of the grid and wandered into the area designated as control. The closest point from the control site to the test area was 200 yards. A previous trapping study on this test site (Reference 2) reported that the mean random travel distance (or average habitat radius) for the beach mouse was 65 yards. The distance traveled on the longest radius observed was over 1000 yards, but this unusual observation was regarded as a freak occurrence. However, it emphasized that a mouse (or mice) could have been contaminated in this way and thus have contaminated pooled samples analyzed for TCDD. Nevertheless, the use of these data as truly control data must be viewed with caution.

The levels of TCDD in the liver of beach mice collected from Grid 1 substantiated bioaccumulation of TCDD, i.e., an accumulation of TCDD in an organism from its environment. In general, levels of TCDD in the livers were no greater than the most concentrated zones of TCDD in the soil. There are no data from this study to support biomagnification of TCDD, i.e., an increase in concentration of TCDD in successive organisms ascending the trophic food chain.

Although the concentration of TCDD on the pelts of beach mice from the test area was only 10 to 15 percent of that in their livers, it was apparent that the mice continually contaminated themselves by repeated movement in and out of their burrows. The soil data substantiating the presence of a zone of TCDD within a short distance of the tunnel entrance suggested recurrent burrowing activity occurred even in established burrows. Likewise, the location of the escape tunnel suggested that even the nest itself may contain detectable levels of TCDD.

Footnote

(2)The value of 0.0368 pound per acre = $4600 \times 8 \times 10^{-6}$. 0.0368 pound of TCDD in 3×10^6 pounds per acre-foot of soil = $0.0368 \times 10^{12}/3 \times 10^6/3$ = 36,800/3 = 12,267 ppt.

c. Histopathology

The only significant lesions seen on histopathologic examinations of 173 adult and fetal beach mice were two instances of moderately severe multifocal, necrotizing, hepatitis and a single mouse with severe venous ectasia of the renal veins in one kidney. All other lesions were of the minor or insignificant type, normally observed in microscopic surveys of large numbers of field animals. The absence of liver lesions (necrosis and porphyria) in animals that had liver levels of TCDD from 20 ppt to 1300 ppt (Table 9) is most significant in view of the massive quantities of both 2,4,5-T and TCDD that must have been applied to the test site. Moreover, a previous study (Reference 2) of this area, which terminated in the summer of 1970, indicated that a significant population of beach mice were inhabiting the test site.

The average life-span of a related species, Peromyscus maniculatus, has been recorded to be less than 5 months and only a few mice lived the full potential of 3 or more years (Reference 13). A single female beach mouse is capable of producing 80 or more young under laboratory conditions with litters being born at approximately 26-day intervals (Reference 15). It is further reported that beach mice on Santa Rosa Island, Florida (within 20 miles of Test Area C-52A), may have produced 10 generations per year (Reference 15). At this frequency the animals collected in 1974 on Grid 1 may be 40 generations removed from the population first noted in 1970. However, a more conservative estimate would be six generations per year (giving a female 60 days to reach sexual maturity), for a total of 24 generations.

It must be stressed that the populations of beach mice noted in 1970 were probably subjected to much greater levels of residual TCDD in the soil than those animals collected in 1974. The absence of pathological signs in mice collected in 1974 indicated that TCDD was neither mutagenic (somatic or germinal) nor carcinogenic in the field at the concentrations noted in Table 30. Since none of the 34 fetuses examined from animals captured on the test grid showed teratogenic defects it must also be concluded the levels of TCDD encountered failed to induce observable developmental defects.

As animals mature, the thymus gland undergoes gradual regression until in the adult it is often found only as a rudimentary structure. Because of the age differences in animals captured from the field, it was impossible to obtain mean thymus weights. In the literature where thymic atrophy is reported following single oral doses of TCDD, young animals of a similar age were used for the laboratory study (Reference 17). Microscopic examination of all thymus gland tissue from both control and TCDD exposed animals further substantiated the lack of thymic lesions in the field situation where animals were exposed, via their burrowing and grooming habits, to soil levels of TCDD as seen in Table 30.

d. Statistical Analysis

Although 32 control and 74 test animals were collected from the field, the population selected for statistical analysis was necessarily smaller (19 and 48, respectively). Removal of all immature mice and pregnant females from the population reduced the range of variability between individuals.

However, it also eliminated the data on control females collected in 1973. This is important to note since the only remaining comparison of liver weights between females were those collected in 1974 from the control site and Grid 1 and which were significant to the 99 percent confidence level. Since the population numbers were low for this comparison (4 versus 4), caution must be used in the interpretation of the results (histological examination did not support differences in liver tissue between control and test animals).

Data on spleen weights between the control and Grid 1 mice were statistically significant (Table 18). It has been reported (Reference 17) that rats given a single treatment with 50 or 100 milligrams of TCDD per killogram of body weight, and examined when the rats became moribund or at time of death, consistently displayed remarkable pathologic changes in the spleen and lymph nodes. These changes consisted of a relative depletion of lymphoid cells and pykosis of the nuclei and degenerative change in the multinucleated megakaryotic type giant cells of the spleen. In the present study, an increase in spleen weight was found in those animals (male and female) collected from the TCDD-exposed field site. However, as with the liver data, histological examination (gross and microscopic) of the spleens did not support differences between the control and test animals. It is interesting to note the magnitude of the standard deviations between spleen weights from the two locations. The magnitude of the differences in the standard deviation may reflect the fluctuations in soil levels of TCDD throughout Grid 1. Thus, not all animals from the test site received the same exposure levels. Because the population numbers were relatively large for this comparison (19 versus 48), and hence a measure of their reliability, the data suggest that the spleen may be the most sensitive organ by which to assess field exposure to TCDD.

The laboratory dusting study confirms one possible method for TCDD contamination of beach mouse livers. Although the liver levels found in the dusting study were not as high as from mice collected from Grid 1, the length of exposure time (28 days) may have accounted for these differences. Moreover, if soil contact is a valid explanation for levels found in the beach mice, then it may also explain the variation in levels of TCDD detected from the racerunner tissues.

This report has reaffirmed that results from laboratory experiments and field studies present widely varying differences in biological organisms' responses to chemicals found in the environment. It should further serve as a caveat regarding conclusions reached from laboratory experiments alone.

SECTION IV

INSECT DENSITY AND DIVERSITY STUDIES ON TEST AREA C-52A, 1973

1. SYNOPSIS OF PREVIOUS ENTOMOLOGICAL RESEARCH ON TA C-52A

During 1970 and 1971, an initial survey of the arthropod populations of Test Area C-52A, Eglin Air Force Base, Florida, was accomplished, and the results were published in Reference 18. The purpose of the survey was to determine arthropod population levels shortly after cessation of massive long-term herbicide applications to the test area.

A sweep net survey of the insects on a 1-mile linear transect of Test Area C-52A resulted in the collection of approximately 1800 specimens belonging to 70 insect families and one non-insect arthropod order. Nineteen of the taxa collected accounted for 86.9 percent of the collection and, of these, three taxa accounted for 46 percent of the collection, i.e., insect families Cicadellidae (leafhoppers) and Lygaeidae (lygaeid plant bugs), and Arachnid order Araneida (spiders). The leafhoppers and plant bugs are herbivores, and the spiders are carnivores. As the plants of the test area were eliminated by the herbicides, those insects which fed specifically upon plants gradually diminished in number. Similarly, the number of predatory insects diminished in the area; however, no direct effects of the herbicide or its residues were observed on any of these arthropod groups.

The present report is the finalization of a study accomplished in June 1973, which was reported in partial form as Section V of Reference 2. The objective of the 1973 study was to duplicate the techniques of the 1971 study as closely as possible in order to evaluate populations along the same surveyed grid line 2 years later. Qualitative and quantitative comparisons are drawn to indicate the changes in vegetative cover of the area and changes in the variety and number of arthropods which had become established on the grid since the aerial dispersal tests were terminated in 1970.

2. MATERIALS AND METHODS

The techniques used in the 1973 study were essentially the same as those outlined in Reference 18. This methodology is summarized below, with any exceptions noted.

This comparative study was based on sweep net collections along north-south sampler row 8 of the 1-square-mile test grid. Sampler row 8 was originally chosen for study due to the diversity of habitats found along that line. The same characteristic was true in 1973. The sweep net study allowed a quantitative comparison of results with the 1971 study, while non-systematic sampling of the grid area would not have lent itself to such analysis. A total of five paired sweep net collections were made on the mornings of June 14, 16 and 18, these dates being approximately 2 years and 2 weeks after the study discussed in Reference 18. The 1-mile grid line which was sampled was divided into 13 transects, each of which was 400 feet long. A given paired sweep was accomplished by traversing across the grid and then back to the starting point. The precise line of vegetation which had been swept during the first half of the paired sampling was not reswept during the return to the starting point. All collections were made by use of a 15-inch-diameter net, and 200 sweeps of the net were accomplished per transect. At the end of each transect, the net contents were emptied into a small paper bag into which an opened vial of ethyl acetate had been placed. These bags were then tightly folded and placed in a large sack which was carried by the collector. After separation of the bag contents in the laboratory, arthropod identifications were carried out to the same taxonomic levels as in the 1971 study. Exceptions to this classification scheme included the listing of acalyptrate muscoid flies as a group and the listing of certain undetermined insects only to order. Full listings of the arthropods found in the two sweep net surveys are given in Table 31 and 32. The acalyptrate muscoid families (three) listed in the 1971 study have been combined in Table 31 in order that statistical comparisons may be more validly accomplished. The undetermined insects in the 1973 study (112 speciments), as listed in Table 32, were for the most part either immature forms or only partial specimens. All identifications were based on information and keys cited in Reference 19.

3. RESULTS AND DISCUSSION

Those arthropods from the 1971 study which were collected in numbers exceeding 1 percent of the total number of specimens are listed in Table 33. Table 34 presents comparable information from the 1973 study. (The various taxa are listed under the heading of families for simplicity.) A comparison of the total number of arthropods found in the two studies indicates that the 1973 collections produced 3.3 times as many animals as the 1971 study. Further analysis of the data indicates that the 1973 survey found great numbers of very small insects as compared to the 1971 study. The majority of the Cicadellidae and Chrysomelidae were very small insects, and the acalyptrate muscoid flies, Psocoptera, Thysanoptera, Sminthuridae, Chalcidoidea and Acarina also fell into this small-to-minute category. This discrepancy in the size of the specimens collected may simply represent a difference in sampling/separation techniques, or it may indicate a true increase/influx of populations of these smaller arthropods as the vegetation and other environmental characteristics of the transects developed since the spray program was terminated.

A comparison of vegetative cover on the test grid between 1971 and 1973 is shown graphically in Figure 46. These data (actually approximations) are derived from Figures III-2 and III-3 of Reference 19. In 11 of the 13 transects there were observed increases in vegetation, while two transects showed no observable changes. Statistical analysis of these vegetative differences (Table 35) showed a mean increase of 16 percent in the vegetative cover of the transects on grid row 8 during the 2 years from 1971 to 1973. This difference in cover was highly significant ($p < 0.001$).

TABLE 31. INSECTS AND ARACHNIDS COLLECTED OR OBSERVED ON TEST AREA C-52A, EGLIN AIR FORCE BASE, FLORIDA, JUNE 1971

GROUP	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
CLASS: ARACHNIDA ORDER: Araneida (Spiders) ORDER: Phalangida (Harvestmen)[a]		335	4	3	25	8	30	188	26	28	7	4	8	2	2
CLASS: INSECTA ORDER: Coleoptera (Beetles) 209 Specimens Collected															
Anthicidae	Antlike Flower Beetles	1											1		
Bruchidae	Seed Beetles	1					1								
Buprestidae[a]	Metallic Wood Borers														
Carabidae	Ground Beetles	4						2	1	1					
Cerambycidae	Long-Horned Beetles	1			1										
Chrysomelidae	Leaf Beetles	43	4	4	1	1	6	5	6	4	2	4	2	4	
Cicindellidae	Tiger Beetles	2				1		1							
Coccinellidae	Lady Beetles	8			1		1		4		2				
Curculionidae	Snout Beetles	10						10							
Dytiscidae[b]	Predacious Diving Beetles														
Elateridae	Click Beetles	84	12	10	15	5	13	10	2	5	1	1		7	3
Gyrinidae[a]	Whirligig Beetles														
Meloidae	Blister Beetles	3							2	1					
Mordellidae	Tumbling Flower Beetles	6						2	4						
Passalidae[b]	Passalid Beetles														

[a]Sighted but not collected in 1971

[b]Sighted or collected in 1970

TABLE 31. INSECTS AND ARACHNIDS COLLECTED OR OBSERVED ON TEST AREA C-52A, EGLIN AIR FORCE BASE, FLORIDA, JUNE 1971 (CONTINUED)

GROUP	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Coleoptera (Beetles) Continued															
Scarabaeidae	Scarab Beetles	2		1					1						
Staphylinidae	Rove Beetles	1										1			
Tenebrionidae	Darkling Beetles	43	2	2	10		2	3	3	4		1	7	3	6
ORDER: Dermaptera (Earwigs)															
Forficulidae	Forficulid Earwigs[a]														
ORDER: Diptera (Flies) 211 Specimens Collected															
Acalyptratae	Acalyptrate Muscoids	31	1		2	2		8	9	1			6	1	1
Anthomyiidae	Anthomyid Flies	14						9	1	2	1	1			
Asilidae	Robber Flies	76	1	4	4	8	11	10	14	7		3	9	3	2
Bibionidae	March Flies	4						4							
Bombiliidae[a,b]	Bee Flies														
Calliphoridae	Blow Flies	2						2							
Chironomidae	Midges	8					4		4						
Culicidae[a,b]	Mosquitoes														
Dolichopodidae	Long-Footed Flies	3						2	1						
Mycetophilidae	Fungus Gnats	18						16	2						
Mydidae	Mydas Flies	23	1	4	3	1	3	2		1	1	4	2	1	
Muscidae	Muscid Flies	17		1			2	9	3				1		1
Pipunculidae	Bigheaded Flies	3					1		2						

TABLE 31. INSECTS AND ARACHNIDS COLLECTED OR OBSERVED ON TEST AREA C-52A, EGLIN AIR FORCE BASE, FLORIDA, JUNE 1971 (CONTINUED)

GROUP	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Diptera (Flies) Continued															
Syrphidae	Flower Flies	9						9							
Tabanidae	Horse Flies, Deer Flies	1							1						
Tachinidae	Tachina Flies	1							1						
Tipulidae	Crane Flies	1				1									
ORDER: Hemiptera (True Bugs) 396 Specimens Collected															
Belostomatidae[b]	Giant Water Bugs														
Coreidae	Coreid Bugs	2			2										
Corimelaenidae	Negro Bugs	5			5										
Cydnidae	Cydnid Bugs	2							1	1					
Gerridae[a]	Water Striders														
Lygaeidae	Lygaeid Bugs	145		4	38	10	19	40	6	19	1		5	1	2
Miridae	Plant Bugs	2	1							1					
Nabidae	Damsel Bugs	72	2	7	8	15	3	6	9	14	2	2	4		
Neididae	Stilt Bugs	1		1											
Nepidae[b]	Water Scorpions														
Notonectidae[b]	Backswimmers														
Pentatomidae	Stink Bugs	82	2	7	22	13	7	5	4	18			4		
Reduviidae	Assassin Bugs	49	1	1	11		14		6	6	1	1	7	1	
Scutelleridae	Shield-Backed Bugs	36		2	3	1	12	1	13	2			2		

TABLE 31. INSECTS AND ARACHNIDS COLLECTED OR OBSERVED ON TEST AREA C-52A, EGLIN AIR FORCE BASE, FLORIDA, JUNE 1971 (CONTINUED)

GROUP	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Homoptera (True Bugs) 360 Specimens Collected															
Aphidae	Plantlice	4				1		1		1	1				
Cercopidae	Spittlebugs	9			1	2	1	1	1	3					
Cicadellidae	Leafhoppers	345	10	30	46	54	41	80	21	29	10	10	10	2	2
Coccidae[a,b]	Scale Insects														
Fulgoridae	Fulgorid Planthoppers	1							1						
Membracidae	Treehoppers	3						2	1						
ORDER: Hymenoptera (Bees, Wasps, Ants) 125 Specimens Collected															
Apidae	Apid Bees	1								1					
Bombidae[b]	Bumble Bees														
Braconidae	Braconid Wasps	11		1	1	1	2	1	3	2					
Chalcididae	Chalcids	2						1	1						
Chrysididae	Cuckoo Wasps	1						1							
Cynipidae	Gall Wasps	2								2					
Formicidae	Ants	12			1	2		6	2			1			
Halictidae	Sweat Bees	25	3	1	2	1		12	5	1					
Ichneumonidae	Ichneumon Wasps	3								1				2	
Megachilidae	Leafcutting Bees	2	1					1							
Mutillidae	Velvet Ants	4		3										1	
Pamphiliidae	Webspinning Sawflies	5	1		1									1	2
Pompilidae	Spider Wasps	6						1		1		1		2	1

TABLE 31. INSECTS AND ARACHNIDS COLLECTED OR OBSERVED ON TEST AREA C-52A, EGLIN AIR FORCE BASE, FLORIDA, JUNE 1971 (CONTINUED)

GROUP	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Hymenoptera (Bees, Wasps, Ants) Continued															
Scoliidae	Scoliid Wasps	1						1							
Sphecidae	Sand Wasps	46	2	2		1	2	4	6	2		1		6	20
Tiphiidae	Tiphiid Wasps	4						4							
Xylocopidae[a]	Carpenter Bees														
ORDER: Isoptera (Termites) Observed Only															
Rhinotermitidae[b]	Subterranean Termites														
ORDER: Lepidoptera (Butterflies and Moths) 16 Specimens Collected															
Danaidae	Milkweed Butterflies														
Geometridae[a,b]	Geometrid Moths														
Lycaenidae[a,b]	Blues and Coppers														
Hesperiidae[a,b]	Skippers														
Microlepidoptera[c]	(Several Families)	8				5	3								
Noctuidae	Owl Moths	1											1		
Nymphalidae[a,b]	Brushfooted Butterflies														
Papilionidae[a,b]	Swallowtail Butterflies														
Pieridae[a,b]	Sulfurs														
Psychidae[b]	Bagworm Moths														
Pyralidae	Pyralid Moths	7						3	1	2	1				

[c]Several families in this group, but identified no further

TABLE 31. INSECTS AND ARACHNIDS COLLECTED OR OBSERVED ON TEST AREA C-52A, EGLIN AIR FORCE BASE, FLORIDA, JUNE 1971 (CONCLUDED)

GROUP	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	K'	LM	MN	NO
ORDER: Neuroptera (Nerve Winged Insects) 9 Specimens Collected															
Chrysopidae[a]	Green Lacewings														
Hemerobaeidae	Brown Lacewings	1					1								
Myrmeleontidae	Antlions	8		1								1	5		1
ORDER: Odonata (Dragonflies and Damselflies) 40 Specimens Collected															
Aeshnidae[b]	Dragonflies														
Coenagrionidae	Damselflies	25					2	21	1		1				
Corduliidae[b]	Dragonflies														
Lestidae[b]	Damselflies														
Libellulidae	Dragonflies	15					1	14							
ORDER: Orthoptera (Grasshoppers and Crickets) 95 Specimens Collected															
Acrididae	Grasshoppers	58	1	6	6	6	8	16	7	4	1	1		1	1
Gryllidae	Crickets	3			1				1	1					
Gryllotalpidae[a,b]	Mole Crickets														
Mantidae	Mantids	4				1	3								
Tettigoniidae	Katydids	23	1		6	4	2	4	5	1					
Trydactylidae	Pygmy Mole Crickets	7						7							
ORDER: Trichoptera (Caddisflies) Observed Only															
Family not determined															
	TOTAL ANTHROPODS	1796	50	95	216	144	195	525	182	166	32	37	74	38	44

(This Table was adapted and modified from Table V-5 of Reference 18.)

TABLE 32. ARTHROPODS COLLECTED ON TEST AREA C-52A, EGLIN AIR FORCE BASE RESERVATION, FLORIDA, JUNE 1973

GROUP	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
CLASS: ARACHNIDA															
ORDER: Araneida	(Spiders)	672	13	40	60	91	108	148	85	52	9	27	27	4	8
ORDER: Acarina	(Mites)	93		3	4	5	5	56	7			2	3		8
CLASS: INSECTA															
ORDER: Coleoptera (Beetles) 910 Specimens Collected															
Anobiidae	Deathwatch Beetles	1		1											
Anthicidae	Antlike Flower Beetles	6						4	1		1				
Carabidae	Ground Beetles	15						14		1					
Chrysomelidae	Leaf Beetles	686	4	3	261	112	46	169	52	5	1	6	14	8	5
Cicindellidae	Tiger Beetles	39	3	6	7	3	10			6	1	1	2		
Cleridae	Checkered Beetles	3		1			1						1		
Coccinellidae	Lady Beetles	16		1		7		2	3	1			1		1
Curculionidae	Snout Beetles	39	3	1	3	7	4	6	10	2			3		
Elateridae	Click Beetles	24		1	3	3	2	3	2	2	1	2	2	1	2
Histeridae	Hister Beetles	3						2							1
Limulodidae	Horseshoe Crab Beetles	1	1												
Meloidae	Blister Beetles	1							1						
Mycetaeidae	Mycetaeid Fungus Beetles	3			1				2						
Phalacridae	Shining Flower Beetles	20			11	7	1		1						
Tenebrionidae	Darkling Beetles	40	4	7	3	2	5	1	1	4	6	1	2	3	1
	Undetermined larvae and adults	13			1		1	6	4			1			
ORDER: Collembola (Springtails) 133 Specimens Collected															
Sminthuridae	Globular Springtails	133			10	9	23	32	35	18	2	3		1	

TABLE 32. ARTHROPODS COLLECTED ON TEST AREA C-52A, EGLIN AIR FORCE BASE RESERVATION, FLORIDA, JUNE 1973 (CONTINUED)

GROUP	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Diptera (Flies) 502 Specimens collected															
Acalyptratae	Acalyptrate Muscoids	295	18	8	23	31	38	67	41	22	8	14	20	5	
Asilidae	Robber Flies	25			2	3	1	4	4	2	5	4			
Bombiliidae	Bee Flies	1							1						
Cecidomyiidae	Gall Midges	12		5	1	5							1		
Ceratopogonidae	Biting Midges	1					1								
Chironomidae	Midges	24			3	2	8	9	1	1					
Culicidae	Mosquitoes	19		1	2	3		8	3	1		1			
Empididae	Empidid Flies	5				4					1				
Muscidae	Muscid Flies	35		2	3	5	5	6	5	3	1	1	1		1
Phoridae	Humpbacked Flies	3		1		1						1			
Pipunculidae	Bigheaded Flies	19	1	2	7			3	3	1			2		
Rhagionidae	Snipe Flies	1													1
Sarcophagidae	Flesh Flies	5	1		2	2									
Syrphidae	Flower Flies	2			1				1						
Tachinidae	Tachind Flies	10		2	4	1	1		1			1			
Tipulidae	Crane Flies	2						2							
	Undetermined Adults	43	1	3	3	6	5	4	7	2	2	4	4	2	
ORDER: Ephemeroptera (Mayflies) 2 Specimens Collected															
Ephemeridae	Burrowing Mayflies	2				1					1				
ORDER: Hemiptera (True Bugs) 715 Specimens Collected															
Corimelaenidae	Negro Bugs	30				8	1	5	14	1			1		
Corizidae	Grass Bugs	17	3	1	1	2	1	4	1						4

TABLE 32. ARTHROPODS COLLECTED ON TEST AREA C-52A, EGLIN AIR FORCE BASE RESERVATION, FLORIDA, JUNE 1973 (CONTINUED)

GROUP	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Hemiptera (True Bugs) Continued															
Lygaeidae	Lygaeid Bugs	127	4	7	24	4	3	33	6	8	13	7	13	4	1
Miridae	Plant Bugs	56	2		6	1	5	21	8	3				6	4
Nabidae	Damsel Bugs	197	5	15	8	13	5	17	6	27	24	6	48	15	8
Neididae	Stilt Bugs	16		1	2	1		4				4	3		1
Pentatomidae	Stink Bugs	13			1	1	1	2	3	1		1	3		
Reduviidae	Assassin Bugs	66			6	8	5	18	9	2	5	4	7	2	
Scutelleridae	Shield-Backed Bugs	106	14	15	18	7	11	2	8	7	3	2	12		7
	Undetermined Nymphs	87	3	13	12	6	1	10	2	4	15	6	11	1	3
ORDER: Homoptera (True Bugs) 1536 Specimens Collected															
Aleyrodidae	Whiteflies	11					4	4	3						
Aphidae	Plantlice	20			1	1	1	15			1	1			
Cercopidae	Spittlebugs	48	5		4	25	1		4		1	4	3		1
Cicadellidae	Leafhoppers	1485	54	84	273	156	94	146	109	99	78	102	119	28	143
Coccoidea	Scale Insects	1							1						
Fulgoridae	Fulgorid Planthoppers	12	2	1	3	1	1	1	1	2					
Membracidae	Treehoppers	9			7			1	1						

TABLE 32. ARTHROPODS COLLECTED ON TEST AREA C-52A, EGLIN AIR FORCE BASE RESERVATION, FLORIDA, JUNE 1973 (CONTINUED)

GROUP	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Hymenoptera (Bees, Wasps, Ants) 495 Specimens Collected															
Andrenidae	Mining Bees	22	2	1	2	1	2		7	6				1	
Bethylidae	Bethylid Wasps	18	2			4	2	7	1	1				1	
Braconidae	Braconid Wasps	30	2		3	4	2	4	11	4					
Chalcidoidea	Chalcid Wasps	97	5	3	10	12	7	13	13	12	5	7	10		
Cynipoidea	Gall Wasps	8	1		4	1	1			1					
Dryinidae	Dryinid Wasps	1					1								
Formicidae	Ants	261	9	14	24	21	24	59	34	20	9	9	32	6	
Halictidae	Sweat Bees	24				9		6	3	2	1		3		
Ichneumonidae	Ichneumon Wasps	4	2								1		1		
Mutillidae	Velvet Ants	5			1	1		1					1		1
Pompilidae	Spider Wasps	2		1								1			
	Undetermined Adults	23		12	2			2	2		1			4	
ORDER: Lepidoptera (Butterflies and Moths) 59 Specimens Collected															
Microlepidoptera (Several Families)		56	1		6	5	5	14	15	2	2	1	4		1
Noctuidae	Owl Moths	3						2		1					
ORDER: Neuroptera (Nerve Winged Insects) 6 Specimens Collected															
Myrmeleontidae	Antlions	6					1			1	1	1	1	1	

TABLE 32. ARTHROPODS COLLECTED ON TEST AREA C-52A, EGLIN AIR FORCE BASE RESERVATION, FLORIDA, JUNE 1973 (CONCLUDED)

GROUP	COMMON NAME	TOTAL SPECIMENS	NUMBER OF SPECIMENS COLLECTED ON TRANSECT												
			AB	BC	CD	DE	EF	FG	GH	HJ	JK	KL	LM	MN	NO
ORDER: Odonata (Dragonflies and Damselflies) 49 Specimens Collected															
Ccenagrionidae	Damselflies	49			3	6	7	20	8	4	1				
ORDER: Psocoptera (Psocids) 275 Specimens Collected															
	Family not determined	275		2	55	25	4	23	10	3	54	21	67	10	1
ORDER: Orthoptera (Grasshoppers and Crickets) 412 Specimens Collected															
Acrididae	Grasshoppers	184	3	6	27	34	7	38	35	22	[illegible]	3	6	1	
Gryllidae	Crickets	52	2	1	4	5	12	1	10	10	2	2	2	1	
Mantidae	Mantids	15	1		2	1		6	1	3	1				
Phasmidae	Walkingsticks	56	1	11	9	5	2	9	11	1	3	1	2		1
Tettigoniidae	Katydids	105	1	3	16	12	15	17	9	4	5	4	12	5	2
ORDER: Thysanoptera (Thrips) 55 Specimens Collected															
	Family not determined	55		1	9	7	2	19	9	1		1	4		2
ORDER: Zoraptera (Zorapterans) 2 Specimens Collected															
Zorotypidae		2					1					1			
	TOTAL ARTHROPODS	5966	173	280	958	597	494	1072	626	375	267	258	448	110	208

TABLE 33. TAXA COLLECTED IN NUMBERS EXCEEDING 1 PERCENT OF THE TOTAL SPECIMENS COLLECTED[a], JUNE 1971

FAMILY	COMMON NAME	NUMBER COLLECTED	PERCENT OF TOTAL	CUM. PERCENT OF TOTAL[b]
Cicadellidae	Leafhoppers	345	19.2	19.2
Araneida (Order)	Spiders	335	18.6	37.8
Lygaeidae	Lygaeid bugs	145	8.1	45.9
Elateridae	Click beetles	84	4.7	50.6
Pentatomidae	Stink bugs	82	4.6	55.2
Asilidae	Robber flies	76	4.2	59.4
Nabidae	Damsel bugs	72	4.0	63.4
Acrididae	Grasshoppers	58	3.2	66.6
Reduviidae	Assassin bugs	49	2.7	69.3
Sphecidae	Sand wasps	46	2.6	71.9
Tenebrionidae	Darkling beetles	43	2.4	74.3
Chrysomelidae	Leaf beetles	43	2.4	76.7
Scutelleridae	Shield-backed bugs	37	2.1	78.8
Acalyptrate Muscoids	Flies	31	1.7	80.5
Coenagrionidae	Damselflies	25	1.4	81.9
Halictidae	Sweat bees	25	1.4	83.3
Mydidae	Mydas flies	23	1.3	84.6
Tettigoniidae	Katydids	23	1.3	85.9
Mycetophilidae	Mycetophilid flies	18	1.0	86.9

[a]Total equals 1796 specimens: 1 percent of the total equals 18 specimens.

[b]Cumulated percent of total is derived by the progressive summation of the figures in the percent of total column.

(This Table is adapted and modified from Table V-3 in Reference 18.)

TABLE 34. TAXA COLLECTED IN NUMBERS EXCEEDING 1 PERCENT OF THE TOTAL SPECIMENS COLLECTED[a], JUNE 1973

FAMILY[b]	COMMON NAME	NUMBER COLLECTED	PERCENT OF TOTAL	CUM. PERCENT OF TOTAL
Cicadellidae	Leafhoppers	1485	24.9	24.9
Chrysomelidae	Leaf Beetles	686	11.5	36.4
Araneida	Spiders	672	11.3	47.7
Acalyptrate Muscoids	Flies	295	4.9	52.6
Psocoptera	Psocids	275	4.6	57.2
Formicidae	Ants	261	4.4	61.6
Nabidae	Damsel bugs	197	3.3	64.9
Acrididae	Grasshoppers	184	3.1	68.0
Sminthuridae	Springtails	133	2.2	70.2
Lygaeidae	Lygaeid bugs	127	2.1	72.3
Scutelleridae	Shield-backed bugs	106	1.8	74.1
Tettigoniidae	Katydids	105	1.8	75.9
Chalcidoidea	Chalcid wasps	97	1.6	77.5
Acarina	Mites	93	1.6	79.1
Reduviidae	Assassin bugs	66	1.1	80.2

[a]Total equals 5966 specimens: 1 percent of the total equals 60 specimens.

[b]As discussed in the text, several of the taxa represent ordinal or super family levels of classification rather than family.

[c]Cumulated percent of total is derived by the progressive summation of the figures in the percent of total column.

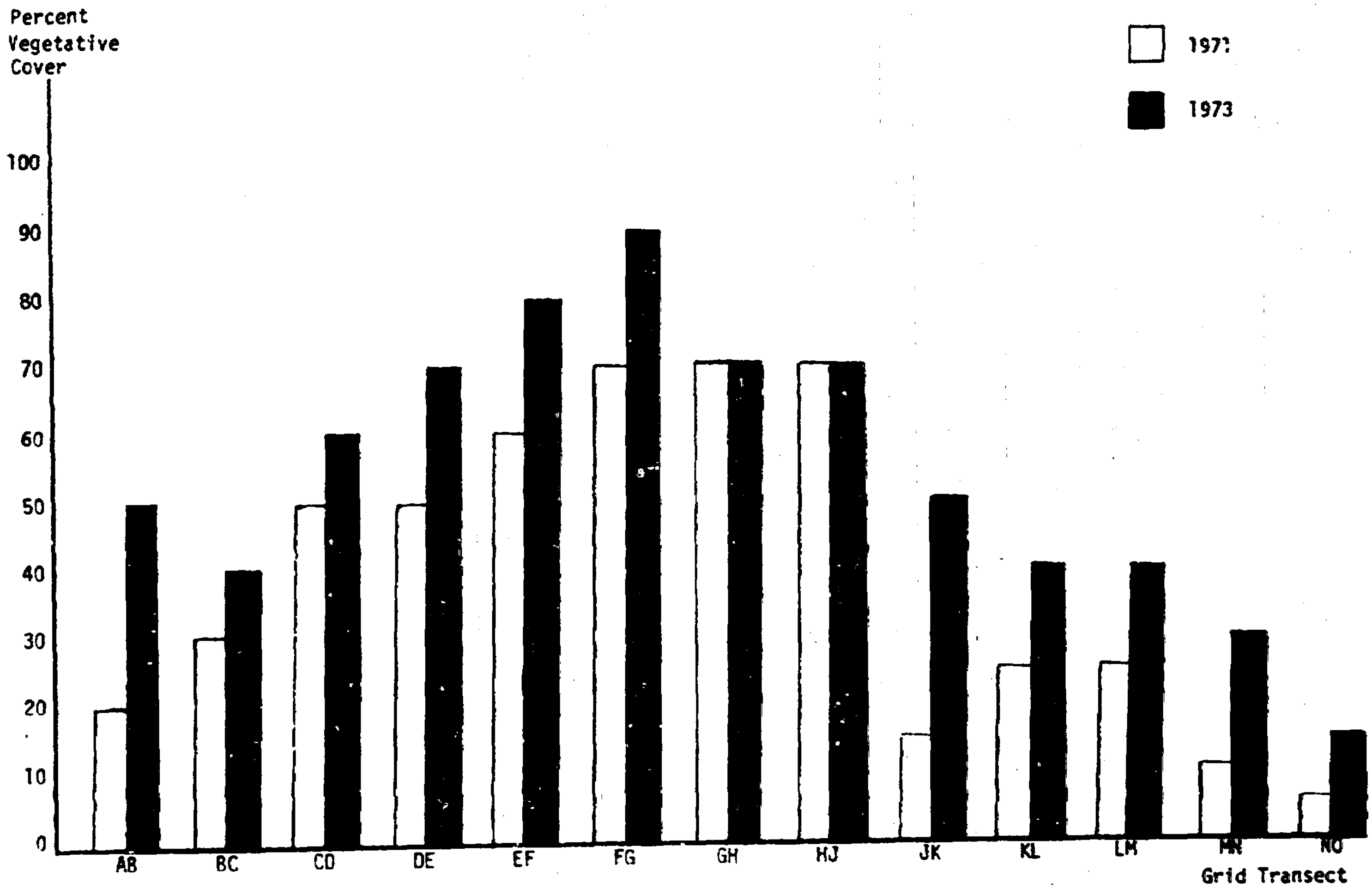

Figure 46. Comparison of Vegetative Cover on Sampler Row 8, TA C-52A, 1971 - 1973

TABLE 35. RESULTS OF STUDENT'S PAIRED t-TESTS, 1971 VERSUS 1973 DATA

DATA COMPARISON[a]	1971 MEAN	1973 MEAN	DIFFERENCE (1973-1971)	STANDARD ERROR OF DIFFERENCE	PROBABILITY[b]
Percent Vegetative Cover	38	54	16	2.8	$p < .001$
Number of Arthropods	138.3	458.8	320.5	55.2	$p < .001$
Number of Arthropod Families	23.2	37.8	14.6	1.9	$p < .001$
Diversity Indices[c]	2.5068	2.5646	0.0581	0.0716	$p > .40$

[a]See Figures 46 through 49

[b]The same significant "p" values result whether these comparisons are considered as one-tailed or two-tailed tests.

[c]Shannon's Diversity Index (References 20 and 21) was used in these calculations:

$$H' = -\sum_i p_i, \text{ where } p_i = N_i/N = \frac{\text{\# specimens per family}}{\text{total \# of specimens}}$$

Shannon's Index of Diversity was used in this study since it is an accepted statistical method in population diversity studies, and inferences were being drawn in this analysis from the sampled populations to apply to the arthropod community of the entire grid. As in the case with any diversity statistic, however, biases do exist, and in this instance it can neither be assumed that the collected arthropod populations were random samples nor that the total number of arthropod species on the grid was known. These biases apply equally, though, to both the 1971 and 1973 studies. The use of natural logarithms rather than base 10 logs merely influences the absolute size of the diversity units and does not affect their relative values.

Due to the fact that plants are used as harborage and as food sources by many terrestrial invertebrates, increases similar to those found in the vegetative community would be expected in the arthropod community. Such increases were found in every transect of the sampled grid row (Figure 47). From 1971 to 1973 the mean number of arthropods collected per transect by the sweep sampling technique increased from 138.3 to 458.5 animals, and this change was again highly significant ($p < 0.001$).

With the increased vegetation in the study area came an increase in the number and variety of ecological niches available to animals. An increase in the variety as well as the number of animals occurred concomitantly with this increase in available niches. The sampled arthropod populations in 1971 and 1973 showed an increase in variety within every transect (Figure 48), and the mean increase per transect was 14.6 families. This increase was again significant at the 0.1 percent level.

The rate of increase in the number of taxonomic varieties of arthropods was not as high as the rate of increase in the actual number of arthropods. This result would be expected for two main reasons. First, the test grid was not depauperate of species at the time of the 1971 study; and even though significant increases in the number of arthropod varieties had occurred by 1973, such increases in the number of families (or species) rapidly level off asymptotically as the grid recovers in the ecological sense. Second, the number of individuals in populations of organisms (especially arthropods) has the capacity to increase exponentially; and while such potential increases in arthropods were not realized in this study (they seldom are), significant population growth did occur and will continue to occur in conjunction with the recovery of the study area. For these reasons, the use of comparative diversity indices can be misleading, since these indices are basically ratio comparisons of the number of taxa versus the number of individuals. Figure 49 illustrates such a comparison of diversity indices for the arthropod populations collected during the 1971 and 1973 studies. Even though there was a significant increase in the number of families (and therefore species) in the sampled area, this increase was offset by the similarly significant increase in the number of specimens collected. Statistical analysis of these indices (Table 35) showed no significant difference ($p < 0.40$) between the 1971 and 1973 study groups. Thus, in this type of situation, comparative analysis should be made on the data which form the basis of a diversity index statistic, and not just on the diversity index itself. It is of interest that there was little change in the diversity of these populations, but reliance in this statistic alone would obscure the fundamental changes actually taking place in the arthropod community of the test grid.

Other population factors which are of interest in comparisons of the 1971 and 1973 data would include the relationship of arthropod population biomass to vegetative cover. While this biomass-vegetation comparison would ideally show a close positive correlation, two factors make the comparison impractical. First, the influence of randomly caught large insects (especially the Orthoptera) on biomass data would be quite confounding until a great deal of repli-

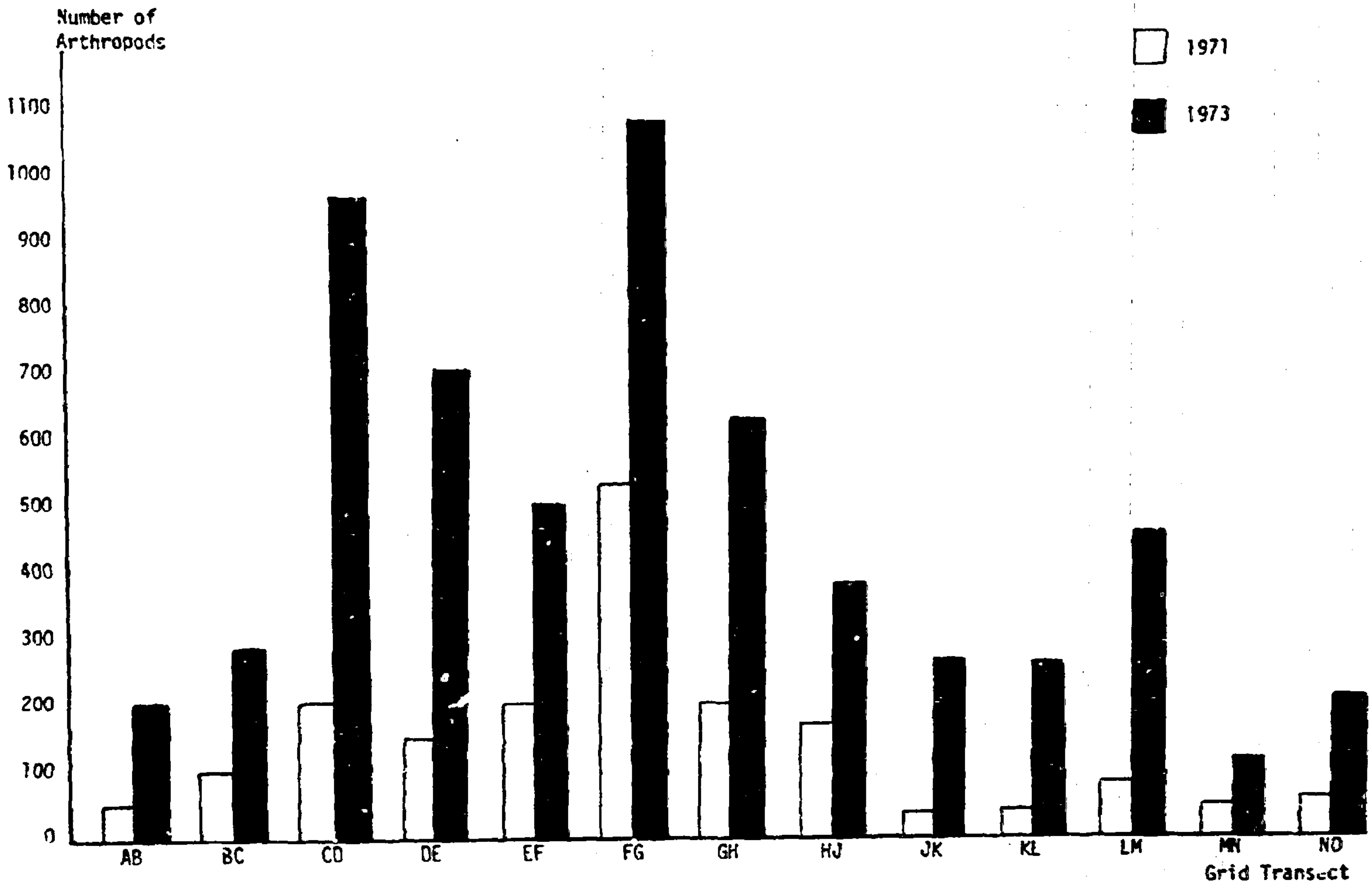

Figure 47. Comparison of Number of Arthropods Collected on Sampler Row 8, TA C-52A, 1971 - 1973

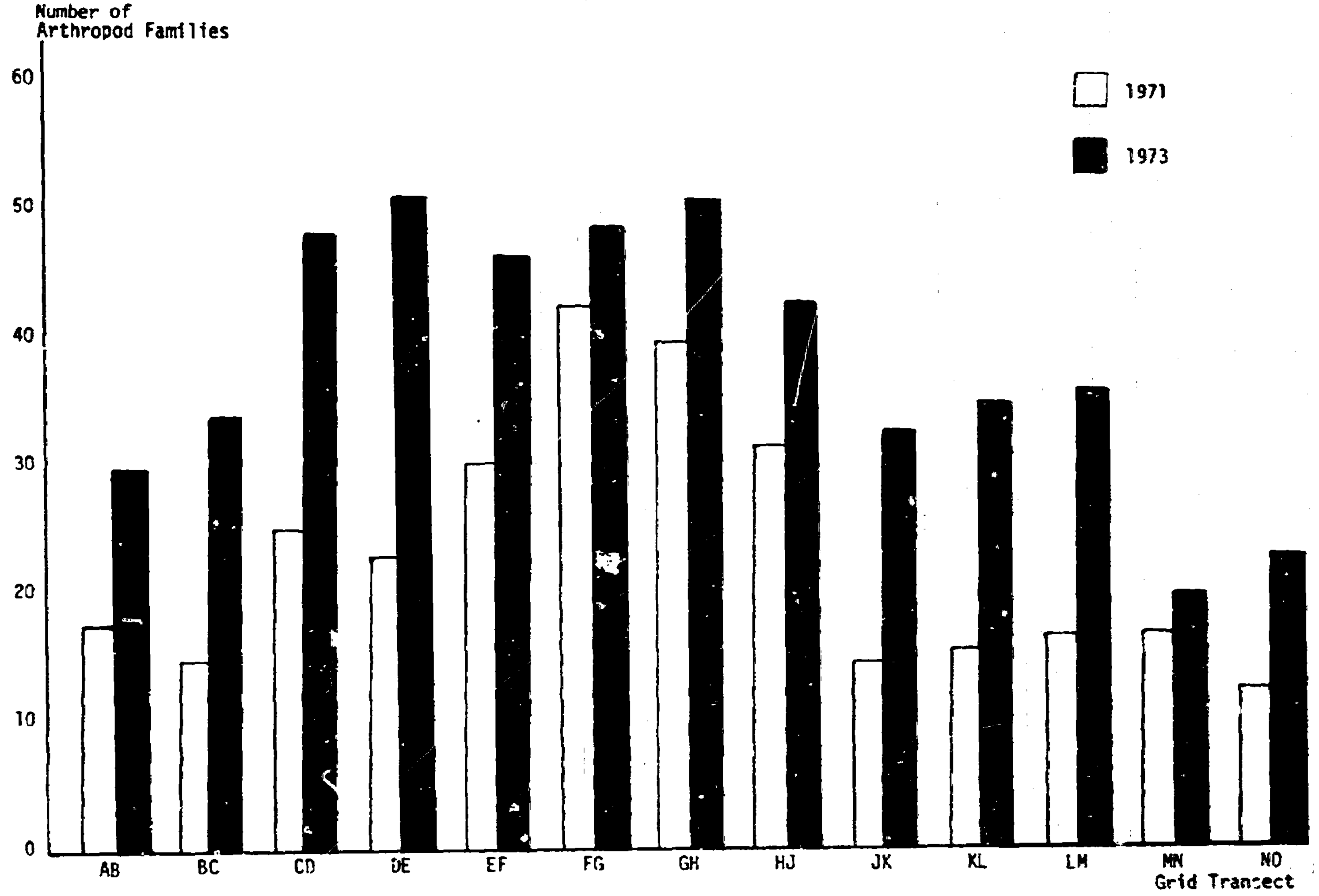

Figure 48. Comparison of Number of Arthropod Families Collected on Sampler Row 8, TA C-52A, 1971 - 1973

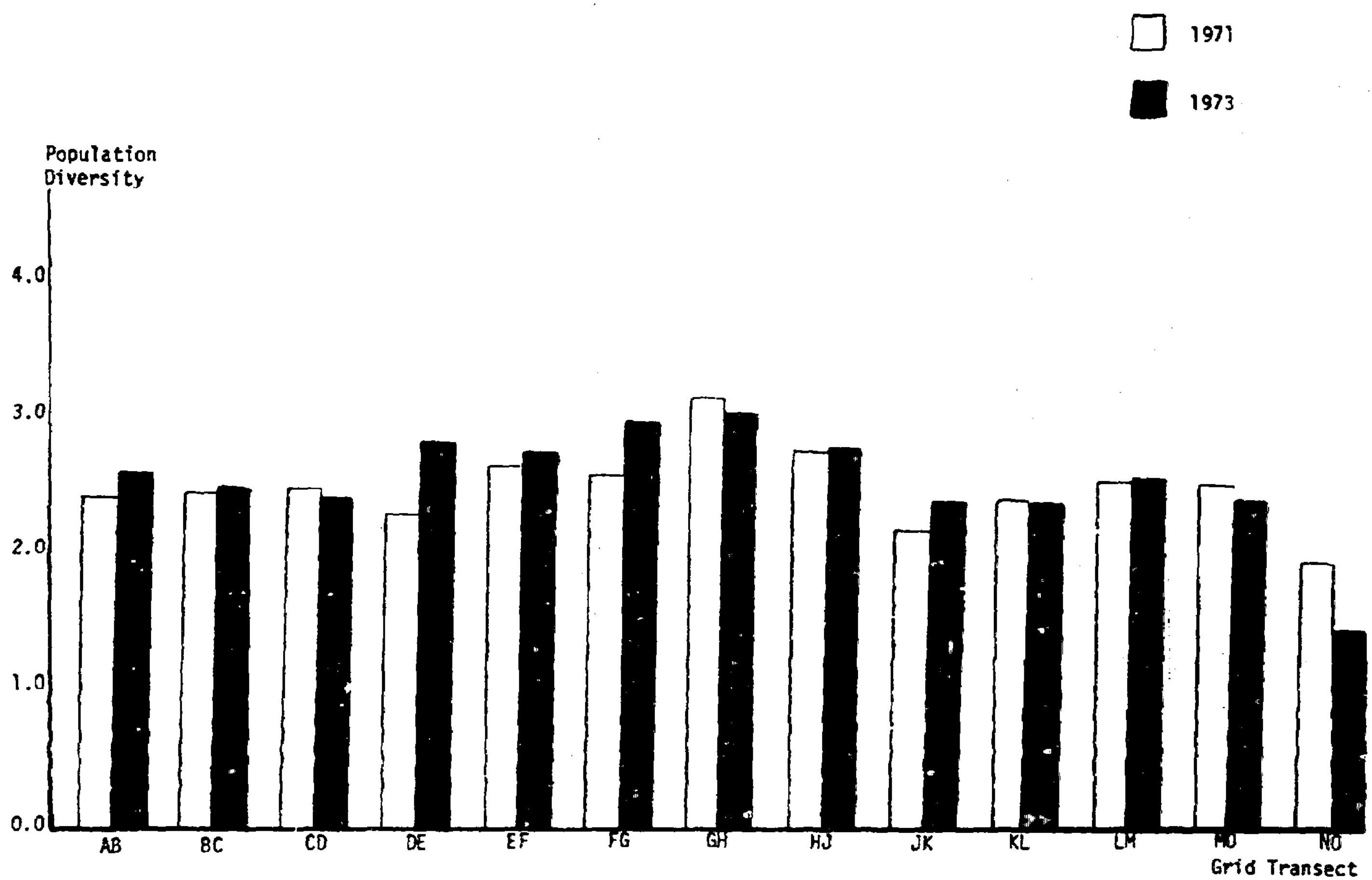

Figure 49. Comparison of Diversity Indices (Shannon's Formula) for Arthropod Populations on Sampler Row 8, TA C-52A, 1971 - 1973

cation had produced a representative sampling of these animals. Second, the factors considered in the 1971 insect survey did not include biomass, so there is no direct basis for comparison with the 1973 survey collections. Many of the topics which were discussed in the 1971 study, including experimental biases of the sweep net sampling technique, factors affecting plant distributions, and plant-insect relationships held equally true in the 1973 study. These subjects will not be discussed further in conjunction with the present comparative analysis since they are available for review in Reference 18.

4. SUMMARY AND CONCLUSIONS

A 1973 sweep net survey of the arthropods of Test Area C-52A on the Eglin Air Force Base Reservation resulted in the collection of 5966 specimens belonging to 71 insect families and two arachnid orders. These totals represent the collections from five paired sweeps taken over a 1-mile section of the test grid. A similar study performed in 1971 produced 1796 specimens, representing 70 insect families and one arachnid order, from five paired sweeps of the same area using the same basic sampling techniques. A much greater number of small to minute insects were taken in the 1973 study. Vegetative coverage of the test area had significantly increased since the 1971 study. Comparison of the results of the two studies also showed significant increases in the number of arthropod specimens and varieties per sampled grid transect, but there was little overall change in calculated community diversity. These results are not unexpected, and the population increases will continue as the test area stabilizes and develops further plant cover, thus allowing a succession of animal populations to invade the recovering habitat.

SECTION V

AQUATIC STUDIES OF TEST AREA C-52A

In order to assess the possible impact of aerial spraying of military herbicide on an aquatic environment under natural conditions, a study of the streams surrounding Test Area C-52A, Eglin Air Force Base, Florida, was initiated in 1969. Permanent collection sites were established on streams that drain the test area and at a pond on the test grid. Fish collections were made at designated sampling sites in 1969, 1970, and 1973 and species diversities compared. These data were presented in an earlier technical report (Reference 2). TCDD analysis of some representative organisms collected in summer 1973 from streams draining Test Area C-52A or in the ponds on the test area were free from TCDD contamination at a lower detection limit of less than 10 ppt. However, since TCDD was found in two silt samples from Trout Creek Bayhead (13 and 10 ppt) and in one silt sample from a pond (11 ppt), it was felt that perhaps an adequate sampling of food chains had not been conducted in these systems.

The purpose of this report is to present the results of the summer 1974 study, and by examination of these data with those obtained previously, draw some conclusions as to the effect of herbicide application on an aquatic ecosystem.

1. METHODS AND MATERIALS

Test Area C-52A is drained by five stream systems: Mullet, Trout, Basin, Grassy, and Rucker Creeks (Figure 50). The combined annual flow from these creeks exceeds 24 billion gallons of water. However, only Mullet, Trout, and Basin Creeks are closely associated with the test grid. The mean daily flow rate for these three streams is given in Table 36. The first studies of fish population diversity indices were initiated in 1969 for Mullet, Trout, Basin, and Little Basin Creeks (Reference 20).

Because of its geographic location to the herbicide dissemination flight paths over the test area, Trout Creek seemed the most likely to receive herbicide residues from the grid area. The headwaters of the stream are at the bottom of steep-sided bayheads adjacent to the edge of the grid and directly in line with the lower extremities of the repeatedly used spray flight path (Figure 50). From its headwaters, the stream flows approximately 2 miles directly south into Choctawhatchee Bay. As the stream nears the bay, it deepens to several feet and has a heavy deposit of leaves and other organic matter on the bottom.

Mullet Creek has portions of its headwaters originating in steep-sided bayheads within 0.5 mile of the west boundary of the spray grid and flows south for approximately 2.5 miles into Choctawhatchee Bay, deepening near its mouth with a heavy deposit of leaves and other organic matter on the bottom (Figure 50).

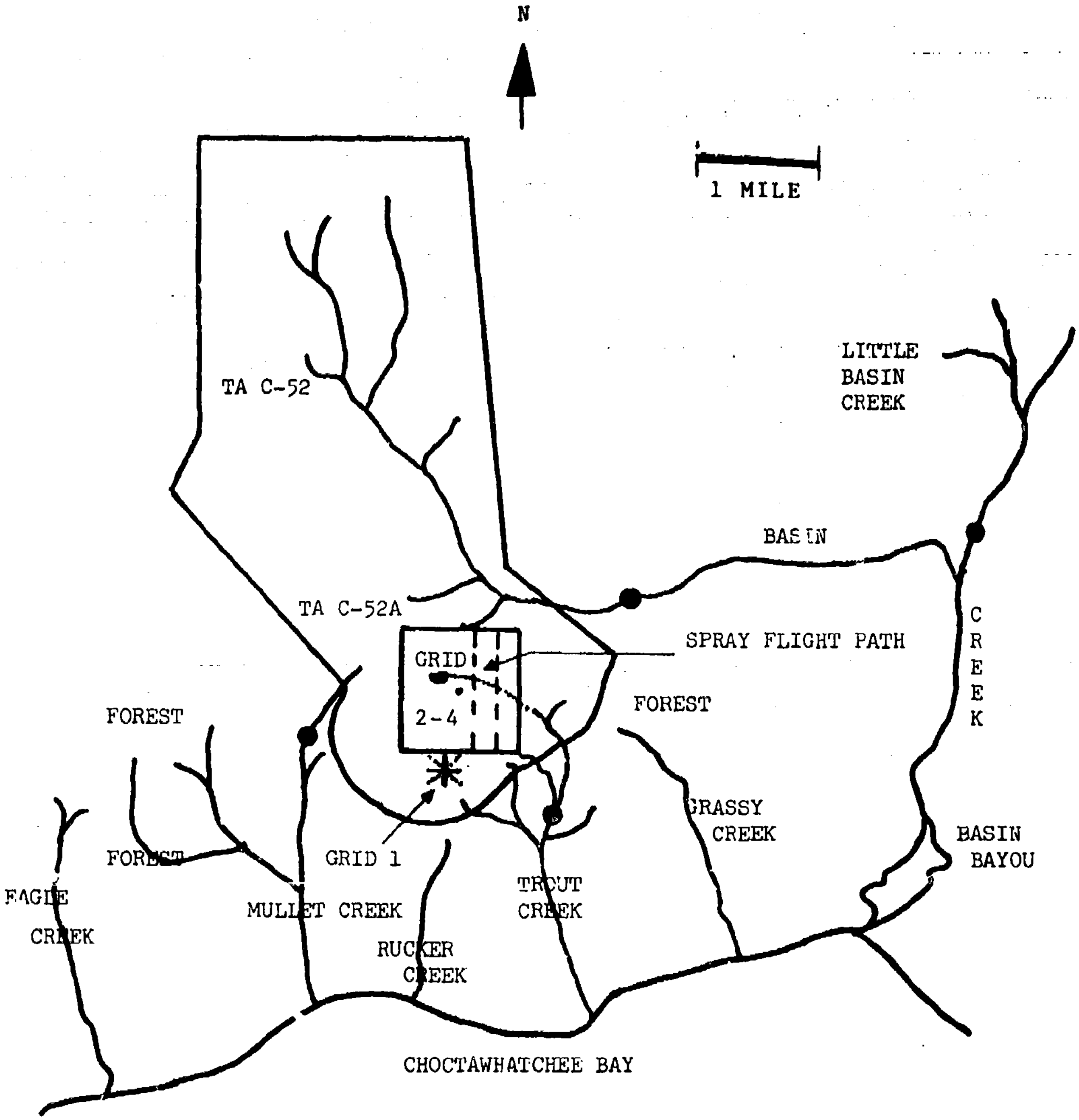

Figure 50. Map of Test Area Showing Streams in Relation to Test Grids and Location of Sampling Stations Used in Species Diversity Studies

TABLE 36. CHARACTERISTICS OF SAMPLING SITES ON STREAMS DRAINING TEST AREA C-52A (REFERENCE 2)

Name	Temperature Range (^oC)	Mean pH	Width (Feet)	Depth (Feet)	Bottom Material	Mean Flow Rate (Gal/Day)
Basin Creek	16-23.5	5.8	to 12	to 4	Sand	39,073,000
Mullet Creek	14-23.0	6.0	to 10	to 2	Sand	3,648,000
Trout Creek	13-23.5	6.1	to 15	to 2	Sand	5,870,000
Little Basin	15-22.5	6.0	to 8	to 3.5	Sand	3,450,000
Creek[a] (Control Stream)						

[a]Does not drain TA C-52A

The headwaters of Basin Creek originate several miles to the north of the test grid. The stream flows southeast within 0.25 mile of the northeast corner of the grid and joins with a small tributary originating at the north margin of the grid, continues east for approximately 3.5 miles and turns south for 2.25 miles emptying into Basin Bayou and Choctawhatchee Bay (Figure 50).

Six sampling stations were initially (1969) established on the three streams: One on Mullet Creek, two on Basin Creek, and three on Trout Creek. The selection of sampling station locations was determined mainly by their accessibility, variation of habitat with the station, and apparent fish populations. Because of the number of stations and time involved, they were not sampled on the same day. However, in the present study (1974) only the four stations shown in Figure 50 were sampled because of positive data on the presence of TCDD in the Trout Creek system.

On each sampling date, observations were made in an effort to detect any gross changes in the population levels of the following selected benthic organisms: crayfish (*Orconectes* sp.), dragonfly naiad (*Gomphus* sp.), freshwater snail (*Neritian* sp.), and an unidentified immature freshwater clam. Observations were also made to detect any morphological effects that may have occurred to eelgrass (*Vallisneria americana*), the only species of vascular aquatic plant common to all stations.

All streams and the test grid pond were collected for an equal period of time and similar sections of streams were sampled over the entire study, in the same manner previously described in Reference 2.

Fishes and other aquatic organisms were collected with a 3.1x1-meter nylon minnow seine. The number of individuals that participated in field work varied from two in 1969 to five in 1974. Specimens to be used for the diversity studies were preserved in a 10 percent formalin solution and transported to the laboratory where they were sorted, identified, and counted. All fishes collected in 1969 and 1974 were accessioned into the University of Alabama Ichthyological Collection (UAIC). Collection data from 1969 through 1974 were arranged in table form and from this, species diversity indices (H') were calculated using Shannon's species diversity formula as discussed by Lloyd, Zar, and Karr (Reference 21), and given as:

$$H' = \sum p_i \operatorname{Log} p_i$$

The number of species and the species diversity value for each collection from each stream were subjected to a single classification analysis of variance for unequal sample sizes as outlined by Sokal and Rohlf (Reference 22) in order to determine if any significant change had occurred in the ichthyofauna of the streams during the 6-year study period.

Samples to be analyzed for TCDD residue were taken from Trout Creek and the grid pond only, since they were most likely to receive concentrations of TCDD which could be incorporated into the food chain. Individuals were collected from areas adjacent to, but not included, in the portion of the streams being used for species diversity studies. For each type of tissue used from the test streams, control samples of the same species were collected from streams outside the study area. Organisms were transported alive to the laboratory where they were killed, dissected, and immediately frozen in clean glass jars. Soil and silt samples were also collected from the bayheads of Trout and Mullet Creeks at locations where erosion of soil had obviously occurred. All samples were analyzed for TCDD by the Interpretive Analytical Services, Dow Chemical U.S.A., Midland, Michigan.

2. RESULTS AND DISCUSSION

A total of 22 species of fishes (Table 37) were collected from the four streams from 1969 through 1974. Twenty-one species were collected at the three test streams (13 species from Mullet Creek, 13 from Trout Creek, and 15 from Basin Creek), while 13 species were collected from the control stream, Little Basin Creek (Table 38). Species lists, number of individuals, and diversity values from each collection for each stream over the study period are given in Tables 39 through 42. Three species, Notropis signipinnis, Erimyzon sucetta, and Lepomis punctatus were collected from the grid pond, but were not used in species diversity calculations. The single specimen of Notropis signipinnis was the first record of this species from the grid pond.

Tables which summarize the results of the single analysis of variance used to compare collection data for each stream are given in Tables 43 through 50. The results indicated that no significant change had occurred in the ichthyofauna of either the test or the control streams during the period of 1969 through 1974. Examination of the cumulative data for each year (Table 37) and for each stream (Tables 39 through 42) shows that there was a general increase in the number of species collected at each station; however, these increments are not significant at the 0.05 or 0.01 levels. It is probable that they reflect a more thorough collection of all available habitats and also an increase in the number of field assistants from 1969 through 1974. This interpretation is supported by the fact that the same upward trend is found in the data for the control stream as well as the test streams.

3. FOOD CHAIN STUDIES

A list of the tissues submitted for TCDD residue analysis and their results are given in Table 51. Of the 34 samples submitted, only six contained detectable amounts of TCDD. All four samples taken from specimens of Lepomis punctatus (Figure 51), the top fish carnivore in the grid pond, contained detectable amounts of TCDD. The lowest concentration (4 ppt) was found in samples of skin and skeletal muscle while the highest concentration

TABLE 37. FISH SPECIES COLLECTED FROM MULLET, TROUT BASIN AND LITTLE BASIN CREEKS FROM 1969 THROUGH 1974

Species	1969	1973	1974
Ichthyomyzon gagei	X	X	X
Anguilla rostrata	X	X	X
Esox americanus	X	X	X
Notropis hypselopterus	X	X	X
Notropis texanus	X	X	X
Erimyzon sucetta			X
Minytrema melanops		X	X
Ictalurus natalis		X	
Noturus funebris			X
Noturus gyrinus			X
Noturus leptacanthus	X	X	X
Aphredoderus sayanus	X	X	X
Gambusia affinis	X	X	X
Heterandria formosa			X
Ambloplites rupestris		X	X
Lepomis gulosus			X
Lepomis punctatus	X	X	X
Micropterus salmoides		X	X
Etheostoma edwini	X	X	X
Percina nigrofasciata	X	X	X
Mugil cephalus		X	
Mugil curema			X

TABLE 38. FISH SPECIES COLLECTED IN TEST AND CONTROL STREAMS FROM 1969 THROUGH 1974. B=BASIN CREEK, M=MULLET CREEK, T=TROUT CREEK, AND LB=LITTLE BASIN CREEK.

Species	Test Streams	Control Streams
Ichthyomyzon gagei	B,M,T	LB
Anguilla rostrata	T	LB
Esox americanus	B,T	LB
Notropis hypselopterus	B,M,T	LB
Notropis texanus	B	
Erimyzon sucetta		LB
Minytrema melanops	B	LB
Ictalurus natalis	T	
Noturus funebris	B,T	
Noturus gyrinus	B	
Noturus leptacanthus	B,M,T	LB
Aphredoderus sayanus	B,M,T	LB
Gambusia affinis	B,M,T	LB
Heterandria formosa	M	
Ambloplites rupestris	B,M,T	
Lepomis gulosus	M	
Lepomis punctatus	B,M,T	LB
Micropterus salmoides	B	LB
Etheostoma edwini	B,M,T	LB
Percina nigrofasciata	B,M,T	LB
Mugil cephalus	M	
Mugil curema	M	

TABLE 39. COLLECTION DATA AND SPECIES DIVERSITY VALUES FOR COLLECTIONS FROM MULLET CREEK FROM 1969 THROUGH 1974

SPECIES	1969 4/4	4/21	5/5	6/27	Total	1973 6/13	6/23	6/27	Total	1974 6/11	6/21	7/1	7/9	Total
Ichthyomyzon gagei	1	-	-	-	1	5	18	10	33	4	3	2	2	11
Notropis hypselopterus	28	35	59	53	175	36	45	76	157	132	113	120	93	450
Noturus leptacanthus	1	-	1	3	5	18	16	11	45	13	21	12	5	51
Aphredoderus sayanus	-	-	-	-	-	1	3	1	5	2	1	1	-	4
Gambusia affinis	13	15	20	8	56	41	51	139	231	140	89	114	150	458
Heterandria formosa	-	-	-	-	-	-	-	-	-	-	-	1	2	3
Ambloplites rupestris	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Lepomis gulosus	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Lepomis punctatus	-	-	-	-	-	2	-	1	3	1	1	-	-	2
Etheostoma edwini	-	-	-	-	-	1	2	5	8	2	6	5	3	16
Percina nigrofasciata	1	1	1	4	7	7	8	4	19	5	8	6	6	25
Mugil cephalus	-	-	-	-	-	-	1	-	-	-	-	-	-	-
Mugil curema	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Total number of species	5	3	4	4	5	8	8	8	9	8	8	8	10	1
Total number of specimens	44	51	81	68	244	111	144	247	502	299	242	261	264	1076
Species diversity value (h')	1.307	1.003	0.988	1.083		2.163	2.249	1.652		1.536	1.789	1.592	1.500	

TABLE 40. COLLECTION DATA AND SPECIES DIVERSITY VALUES FOR COLLECTIONS FROM TROUT CREEK FROM 1969 THROUGH 1974

SPECIES	1969 4/4	4/21	5/5	6/27	Total	1973 6/12	6/21	6/25	Total	1974 6/11	6/17	6/28	7/9	Total
Ichthyomyzon gagei	-	-	-	-	-	10	13	15	38	4	4	2	2	12
Anguilla rostrata	-	-	-	1	1	-	-	-	-	-	-	-	-	-
Esox americanus	-	-	-	-	-	-	4	2	6	-	2	-	-	2
Notropis hypselopterus	90	40	80	39	249	55	69	96	220	213	176	123	119	631
Ictalurus natalis	-	-	-	-	-	1	-	-	1	-	-	-	-	-
Noturus funebris	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Noturus laptacanthus	2	1	-	-	3	5	12	11	28	3	7	3	-	13
Aphredoderus sayanus	-	-	-	-	-	1	1	3	5	1	1	-	2	4
Gambusia affinis	2	2	40	16	60	20	14	46	80	25	20	11	19	75
Ambloplites rupestris	-	-	-	-	-	-	1	-	1	1	-	-	-	1
Lepomis punctatus	-	1	1	4	6	3	2	3	8	1	1	3	-	5
Etheostoma edwini	1	-	1	2	4	5	10	2	17	4	4	6	4	18
Percina nigrofasciata	6	13	4	-	23	25	21	19	65	5	17	8	18	48
Total number of species	5	5	5	5	7	9	10	9	11	9	9	8	7	11
Total number of specimens	101	57	126	62	346	125	147	197	469	257	232	156	165	811
Species diversity value(H')	0.680	1.219	1.210	1.436		22.312	2.429	2.155		1.018	1.365	1.288	1.380	

TABLE 41. COLLECTION DATA AND SPECIES DIVERSITY VALUES FOR COLLECTIONS FROM BASIN CREEK FROM 1969 THROUGH 1974

SPECIES	1969					1973				1974				
	4/11	4/25	5/13	6/16	Total	6/15	6/23	6/26	Total	6/12	6/18	6/26	7/3	Total
Ichthyomyzon gagei	-	-	1	-	1	2	2	1	5	-	3	2	-	5
Esox americanus	-	-	-	1	1	-	-	-	-	2	-	-	-	2
Notropis hypselopterus	83	135	100	47	365	87	23	96	206	168	50	52	32	302
Notropis texanus	-	-	1	-	1	4	-	-	4	2	1	-	1	4
Minytrema melanops	-	-	-	-	-	1	-	-	1	-	-	-	-	-
Noturus funebris	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Noturus gyrinus	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Noturus leptacanthus	-	1	-	-	1	3	5	5	13	3	2	-	-	5
Aphredoderus sayanus	-	-	-	8	8	6	5	6	17	7	1	1	7	16
Gambusia affinis	2	6	27	15	50	49	18	65	132	42	50	25	24	141
Ambloplites rupestris	-	-	-	-	-	-	-	-	-	3	-	1	-	4
Lepomis punctatus	-	-	-	-	-	1	2	4	7	2	1	-	1	4
Micropterus salmoides	-	-	-	-	-	-	-	-	-	-	1	-	1	2
Etheostoma edwini	2	2	2	-	6	7	4	12	23	8	21	6	3	38
Percina nigrofasciata	3	2	4	1	10	38	10	34	82	33	19	29	12	93
Total number of species	4	5	6	5	9	10	8	8	10	11	10	79	9	14
Total number of specimens	90	146	135	72	443	198	69	223	490	271	150	116	82	618
Species diversity value	0.515	0.513	1.130	1.400		2.150	2.521	2.085		1.831	2.349	1.936	2.067	

TABLE 42. COLLECTION DATA AND SPECIES DIVERSITY VALUES FOR COLLECTIONS FROM LITTLE BASIN CREEK FROM 1969 THROUGH 1974

SPECIES	1969					1973				1974				
	4/11	4/25	5/13	6/13	Total	6/20	6/25	6/22	Total	6/13	6/18	6/26	7/3	Total
Ichthyomyzon gagei	-	1	1	-	2	6	5	4	15	-	3	-	1	4
Anguilla rostrata	-	-	-	-	-	1	-	-	1	-	1	1	-	2
Esox americanus	-	1	-	-	1	1	1	2	4	3	1	2	1	7
Notropis hypselopterus	51	125	82	62	320	41	87	49	177	129	86	67	65	347
Erimyzon sucetta	-	-	-	-	-	-	-	-	-	1	-	-	-	1
Minytrema melanops	-	-	-	-	-	-	-	-	-	2	-	-	-	2
Noturus leptacanthus	6	2	1	3	12	6	7	2	15	13	15	3	5	36
Aphredoderus sayanus	-	-	1	-	1	5	6	2	13	4	2	4	-	10
Gambusia affinis	1	4	-	-	5	-	2	-	2	-	-	-	1	1
Lepomis punctatus	-	-	-	-	-	5	2	1	8	6	4	3	1	14
Micropterus salmoides	-	-	-	-	-	-	-	1	1	-	-	-	-	-
Etheostoma edwini	1	2	5	1	9	5	9	6	20	7	7	4	7	25
Percina nigrofasciata	5	5	-	2	12	6	18	4	28	18	17	16	7	58
Total number of species	5	7	5	4	8	9	9	9	11	9	9	8	8	12
Total number of specimens	64	140	90	68	362	76	137	71	284	183	136	100	88	507
Species diversity value(H')	1.056	0.741	0.570	0.559		2.287	1.880	1.747		1.628	1.829	1.665	1.433	

TABLE 43. SINGLE CLASSIFICATION ANALYSES OF VARIANCE ON THE NUMBER OF SPECIES COLLECTED FROM MULLET CREEK FROM 1969 THROUGH 1974

SOURCE OF VARIATION	dF	SS	MS	Fs
AMONG GROUPS	2	47.20	23.60	0.051 ns
WITHIN GROUPS	8	3699.00	462.40	
TOTAL	10	3746.20		
$F_{0.05(2,8)}=4.46$ $F_{0.01(2,8)}=8.65$				

TABLE 44. SINGLE CLASSIFICATION ANALYSES OF VARIANCE ON THE SPECIES DIVERSITY VALUES (H') CALCULATED FROM FISH COLLECTIONS FROM MULLET CREEK FROM 1969 THROUGH 1974

SOURCE OF VARIATION	dF	SS	MS	Fs
AMONG GROUPS	2	1.49	0.74	0.080
WITHIN GROUPS	8	69.68	8.71	
TOTAL	10	71.17		
$F_{0.05(2,3)}=4.46$ $F_{0.01(2,8)}=8.65$				

TABLE 45. SINGLE CLASSIFICATION ANALYSES OF VARIANCE ON THE NUMBER OF SPECIES COLLECTED FROM TROUT CREEK FROM 1969 THROUGH 1974

SOURCE OF VARIATION	dF	SS	MS	Fs
AMONG GROUPS	2	37.1	18.6	0.090 ns
WITHIN GROUPS	8	1639.4	205.0	
TOTAL	10	1676.5		
$F_{0.05(2,8)}=4.46$ $F_{0.01(2,8)}=8.65$				

TABLE 46. SINGLE CLASSIFICATION ANALYSES OF VARIANCE ON THE SPECIES DIVERSITY VALUES (H') CALCULATED FROM FISH COLLECTIONS FROM TROUT CREEK FROM 1969 THROUGH 1974

SOURCE OF VARIATION	dF	SS	MS	Fs
AMONG GROUPS	2	2.66	1.33	0.160 ns
WITHIN GROUPS	8	66.16	8.27	
TOTAL	10	68.82		
$F_{0.05(2,8)}=4.46$ $F_{0.01(2,8)}=8.65$				

TABLE 47. SINGLE CLASSIFICATION ANALYSES OF VARIANCE ON THE NUMBER OF SPECIES COLLECTED FROM BASIN CREEK FROM 1969 THROUGH 1974

SPECIES	dF	SS	MS	Fs
AMONG GROUPS	2	41.30	20.65	0.092 ns
WITHIN GROUPS	8	1777.40	222.20	
TOTAL	10	1818.70		
$F_{0.05(2,8)}=4.46$	$F_{0.01(2,8)}=8.65$			

TABLE 48. SINGLE CLASSIFICATION ANALYSES OF VARIANCE ON THE SPECIES DIVERSITY VALUES (H') CALCULATED FROM FISH COLLECTIONS FROM BASIN CREEK FROM 1969 THROUGH 1974

SPECIES	dF	SS	MS	Fs
AMONG GROUPS	2	2.56	1.28	0.10 ns
WITHIN GROUPS	8	96.23	12.02	
TOTAL	10	98.79		
$F_{0.05(2,8)}=4.46$	$F_{0.01(2,8)}=8.65$			

TABLE 49. SINGLE CLASSIFICATION ANALYSES OF VARIANCE ON THE NUMBER OF SPECIES COLLECTED FROM LITTLE BASIN CREEK FROM 1969 THROUGH 1974

SOURCE OF VARIATION	dF	SS	MS	Fs
AMONG GROUPS	2	31.0	15.5	0.073
WITHIN GROUPS	8	1683.7	210.5	
TOTAL	10	1714.7		
$F_{0.05(2,8)}=4.46$		$F_{0.01(2,8)}=8.65$		

TABLE 50. SINGLE CLASSIFICATION ANALYSES OF VARIANCE ON THE SPECIES DIVERSITY VALUES (H') CALCULATED FROM FISH COLLECTIONS FROM LITTLE BASIN CREEK FROM 1969 THROUGH 1974

SOURCE OF VARIATION	DF	SS	MS	Fs
AMONG GROUPS	2	2.99	1.49	0.19 ns
WITHIN GROUPS	8	61.85	7.73	
TOTAL	10	64.84		
$F_{0.05(2,8)}=4.46$		$F_{0.01(2,8)}=8.65$		

TABLE 51. SAMPLES SUBMITTED FOR TCDD RESIDUE ANALYSIS AND THE RESULTS OBTAINED. PPT = PARTS PER TRILLION AND ND = NOT DETECTED.

NO.	TISSUE	LOCATION	GRAMS SUBMITTED	TCDD ppt	LIMITS OF DETECTION, ppt
1.	Whole insect larvae	Trout Creek	5.71	nd	8
2.	*Erimyzon sucetta* skin	Grid Pond	11.03	nd	10
3.	Whole snails	Trout Creek	99.95	nd	2
4.	Erimyzon sucetta muscle and gut	Grid Pond	32.34	nd	20
5.	Whole diving beetles	Grid Pond	10.38	nd	30
6.	Lepomis punctatus skin	Grid Pond	65.10	4	
7.	*Lepomis punctatus* gonads	Grid Pond	23.36	18	
8.	*Lepomis punctatus* muscle	Grid Pond	117.21	4	
9.	*Lepomis punctatus* gut	Grid Pond	18.78	85	
10.	*Esox americanus* muscle	Trout Creek	65.62	nd	2
11.	*Esox americanus* liver	Trout Creek	5.85	nd	15
12.	*Esox americanus* skin	Trout Creek	24.39	nd	10
13.	Crayfish muscle and viscera	Trout Creek	4.62	nd	4
14.	*Minytrema melanops* muscle	Control	47.19	nd	3
15.	*Minytrema melanops* skin	Control	10.36	nd	4
16.	*Minytrema melanops* gut	Control	6.01	nd	8
17.	*Erimyzon sucetta* skin	Control	8.72	nd	9
18.	*Lepomis punctatus* whole	Trout Creek	55.76	nd	2
19.	*Erimyzon sucetta* muscle	Control	34.25	nd	3
20.	*Erimyzon sucetta* gut	Control	4.04	nd	5
21.	*Lepomis punctatus* skin	Control	38.06	nd	2
22.	*Lepomis punctatus* gonads	Control	3.50	nd	10
23.	*Lepomis punctatus* muscle	Control	68.28	nd	2
24.	*Lepomis punctatus* gut	Control	12.75	nd	4
25.	Crayfish muscle	Control	12.23	nd	4
26.	Crayfish viscera	Control	8.30	nd	7
27.	*Gambusia affinis*, bodies without heads, tails, and viscera	Trout Creek	10.39	12	
28.	*Notropis hypselopterus* gut	Trout Creek	4.66	12	
29.	*Notropis hypselopterus*, bodies without heads, tails and viscera	Trout Creek	55.32	nd	3
30.	*Esox americanus* skin	Control	16.75	nd	8
31.	*Esox americanus* muscle	Control	51.82	nd	2
32.	*Esox americanus* gut	Control	10.90	nd	3
33.	*Rana pipiens* tadpole muscle and gut	Grid Pond	10.09	nd	20

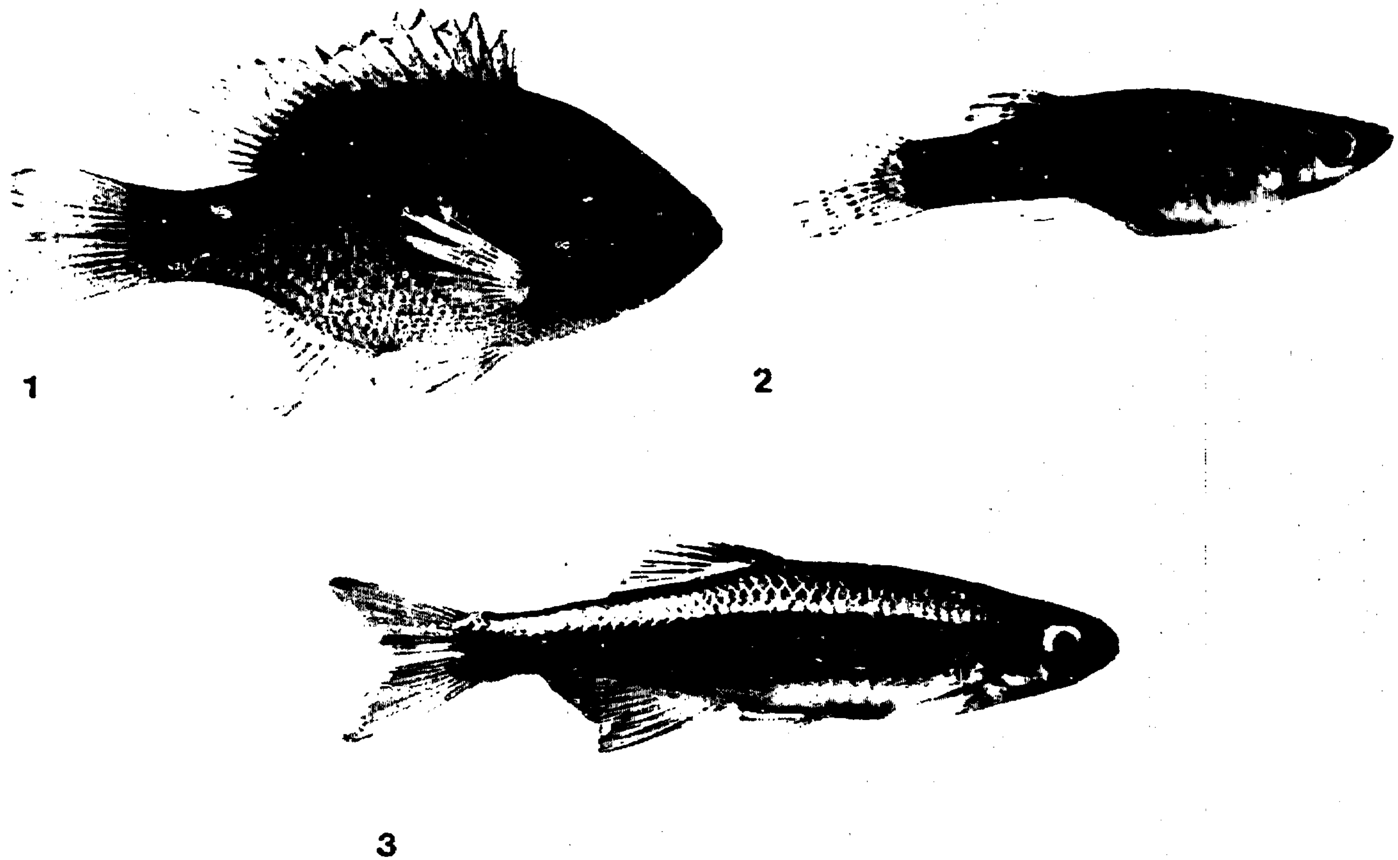

Figure 51. The Three Fish Species Collected from Waters Draining TA C-52A and Found to Contain Detectable Concentrations of TCDD: 1. Lepomis punctatus (4-6 Inches), 2. Gambusia affinis (0.5-1.5 Inches), 3. Notropis hypselopterus (1-3 Inches).

(85 ppt) was found in the visceral mass. This seems to indicate that the TCDD concentrations in the body are probably due to its ingestion and subsequent absorption by the tissues. The concentration of 18 ppt TCDD in the gonads (male and female together) is noteworthy since it is four times that in the skeletal muscle. Because of the insufficient amount of visceral mass obtained from the Lepomis specimens from the grid pond, stomach analysis was not performed on this species; however, the stomach of other individuals of Lepomis from Trout Creek were found to contain large amounts of dipteran, coleopteran, and hymenopteran parts which indicated that these fishes were eating mostly terrestrial insects. Smaller amounts of vegetable material and unidentifiable organic matter were also found. Other tissues submitted for residue analysis from the grid pond included lake chubsucker (Erimyzon sucetta) skin, muscle and visceral mass, and diving beetles, but no TCDD was found in any of these samples.

The analysis for TCDD in soil and silt from an x-section of Trout and Mullet Creek Bayheads is shown in Figure 52. Note that only in Trout Creek were significant concentrations (35 ppt) of TCDD found in the silt of the aquatic system. This reconfirms earlier findings (Reference 2) of TCDD in Trout Creek and further substantiates the long persistence time of TCDD in the ecosystem. The source of TCDD in Trout Creek Bayhead is thought to have been erosion of soil from Grid 1, a 92-acre area sprayed with 87,186 pounds of 2,4,5-T herbicide in the 1962-1964 spray-equipment testing program.

Detectable concentrations of TCDD (12 ppt) were found in two fish species from Trout Creek, Notropis hypselopterus (sailfin shiner), and Gambusia affinis (mosquito fish). The mosquito fish (Figure 51) is a slackwater species that eats predominantly mosquito larvae and pupa, copepods, and small fish (Reference 23). Because of their small size (usually less than 20 mm standard length), individuals of this species were submitted whole except for heads and tails which were removed. Two samples of Notropis hypselopterus were submitted, one containing viscera only and the other comprising bodies less heads, viscera and caudal fins. The viscera sample was found to contain 12 ppt TCDD while in the body samples none was detected.

The extent to which TCDD is involved in the aquatic ecosystems of the streams surrounding TA C-52A is uncertain except to say that it is present in detectable levels in certain fish species from both Trout Creek and the grid pond. Entry into the body is probably through ingestion of food, and although the specific dietary components containing the TCDD is unknown, mosquito and terrestrial insects are likely candidates. The lowest concentrations of TCDD were found in samples of skin and muscle while the highest concentration was found in the visceral mass. A higher concentration was found in the gonad sample than in skin and muscle of Lepomis which may indicate that TCDD is concentrated more in some organs than in others.

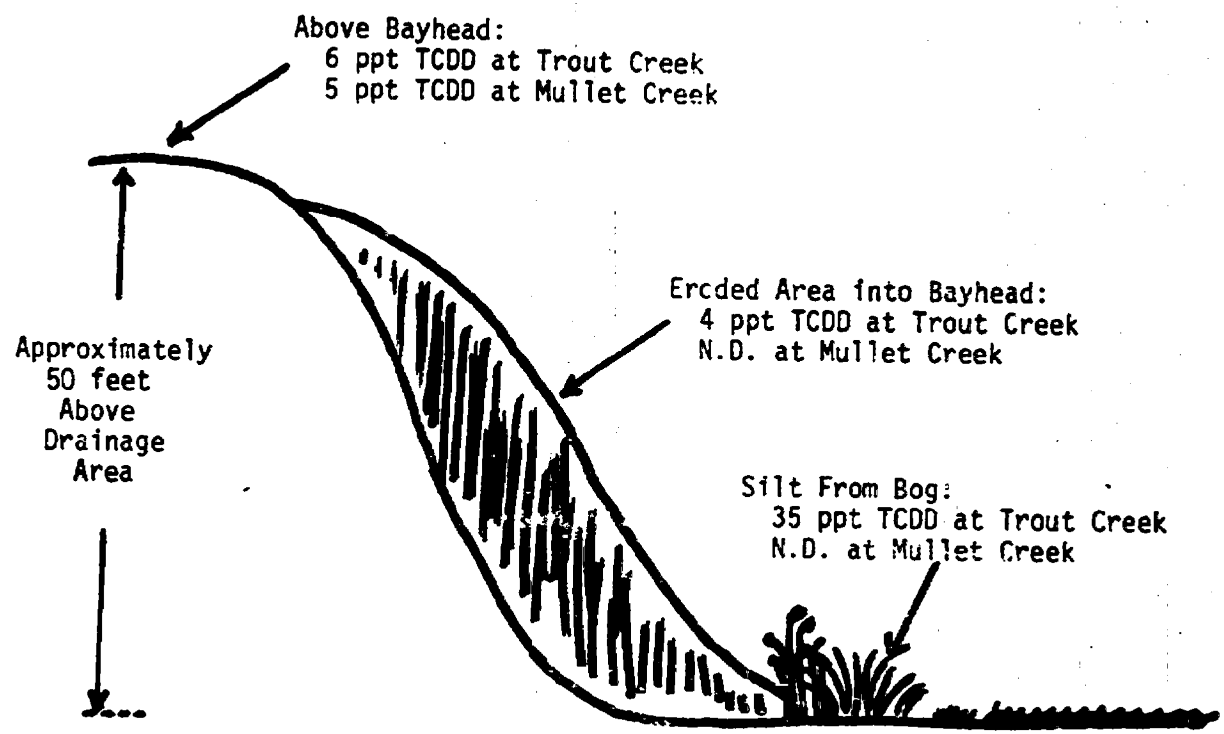

Figure 52. Concentration of TCDD (Parts Per Trillion) in an X-Section of the Bayheads of Trout and Mullet Creeks; Located Approximately One-Quarter Mile from Edge of Test Grid 1, East or West, Respectively

4. CONCLUSIONS

Based on the statistical analysis of collection data presented herein, there is no evidence to indicate there has been any significant change in the composition of the ichthyofauna of the streams surrounding TA C-52A between 1969 and 1974. TCDD is found in both Trout Creek and the grid pond where it is present at various concentrations in individuals of *Notropis hypselopterus*, *Gambusia affinis*, and *Lepomis punctatus*. The food source of these species was not precisely determined due to insignificant study material; however, based on literature records and partial stomach analyses, it appears that they were eating mostly terrestrial insects. The occurrence of TCDD in other aquatic organisms in Trout Creek and the grid pond is unknown at this time.

REFERENCES

1. D. B. Ward, Ecological Records on Eglin AFB Reservation -- The First Year, AFATL-TR-67-157 (Eglin Air Force Base, Florida: Air Force Armament Laboratory, October 1967).

2. A. L. Young, Ecological Studies on a Herbicide-Equipment Test Area (TA C-52A), Eglin AFB Reservation, Florida, AFATL-TR-74-12 (Eglin Air Force Base, Florida: Air Force Armament Laboratory, January 1974).

3. Defoliant History of Test Area C-52A, Working Papers (Eglin Air Force Base, Florida: Vitro Corporation of America and Armament Development and Test Center, December 1969).

4. "Supplement to Working Papers on Defoliant History of Test Area C-52A" (Eglin Air Force Base, Florida: Air Force Armament Laboratory, March 1971).

5. D. B. Ward, Ecological Records on Eglin AFB Reservation -- The Second Year, AFATL-TR-68-147 (Eglin Air Force Base, Florida: Air Force Armament Laboratory, 1968).

6. H. H. Hunter and B. M. Agerton, Annual Diameter Growth of Conifers Adjacent to Eglin Reservation Test Area C-52A as Related to the Testing of Defoliant Spray Equipment, AFATL-TR-71-52 (Eglin Air Force Base, Florida: Air Force Armament Laboratory, 1971).

7. T. T. Sturrock and A. L. Young, A Histological Study of Yucca Filamentosa L. From Test Area C-52A, Eglin Reservation, Florida, AFATL-TR-70-125 (Eglin Air Force Base, Florida: Air Force Armament Laboratory, 1970).

8. J. H. Hunter and A. L. Young, Vegetative Succession Studies on a Defoliant-Equipment Test Area, Eglin AFB Reservation, Florida, AFATL-TR-72-31 (Eglin Air Force Base, Florida: Air Force Armament Laboratory, 1972).

9. F. F. Bartleson, D. D. Harrison, and C. I. Miller, A Survey of Trees on a Herbicide Treated Test Area, Eglin AFB, Florida, AFATL-TR-74-190 (Eglin Air Force Base, Florida: Air Force Armament Laboratory, 1974).

10. "Percentage Frequencies of Temperatures and Dewpoints at the 6-Foot Level, TA C-52A" (Eglin Air Force Base, Florida: ADTC Computer Sciences Laboratory, 1973). (Period of record is January 1963 through October 1969.)

11. B. D. Pate, R. C. Voight, P. J. Lehn, and J. H. Hunter, Animal Survey of Test Area C-52A, Eglin AFB Reservation, Florida, AFATL-TR-72-72 (Eglin Air Force Base, Florida: Air Force Armament Laboratory, April 1972).

12. "Report of the Advisory Committee on 2,4,5-T to the Adminstrator of the Environmental Protection Agency," 7 May 1971.

REFERENCES (CONCLUDED)

13. W. F. Blair, "Population Structure, Social Behavior, and Environmental Relations in Natural Populations of the Beach Mouse (Peromyscus polionotus leucephalus)," Laboratory of Vertebrate Biology, University of Michigan, No. 48 (Ann Arbor: Univ of Mich, June 1951), pp 1-47.

14. J. G. Zinkl, J. G. Voss, J. A. Moore, and B. N. Gupta, "Hematologic and Clinical Chemistry Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Laboratory Animals," Environmental Health Perspectives Experimental Issue, No. 5 (Sep 1973).

15. W. W. Bowen, Variation and Evolution of Gulf Coast Populations of Beach Mice, Peromyscus polionotus, Bulletion of the Florida State Museum, 12(1) (Gainesville: Univ of Fla, 1968), pp 1-91.

16. A. H. Benton and W. E. Werner, Jr., Field Biology and Ecology (New York: McGraw Hill, 1966).

17. B. N. Gupta, J. G. Vos, J. A. Moore, et al, "Pathological Effects of 2,3,7,8-Tetrachlorodibenzo-p-dioxin in Laboratory Animals," Environmental Health Perspectives 5, Experimental Issue No. 5 (Sep 1973), pp 125-140.

18. S. M. Valder, Insect Density and Diversity Studies on Test Area C-52A, Eglin AFB Reservation, Florida, AFATL-TN-72-4 (Eglin Air Force Base, Florida: Air Force Armament Laboratory, January 1972).

19. D. J. Borror and D. M. DeLong, An Introduction to the Study of Insects (New York: Rinehart, 1952).

20. P. J. Lehn, A. L. Young, N. A. Hanme, and B. C. Wolverton, Studies to Determine the Presence of Artificially Induced Arsenic Levels in Three Freshwater Streams and Its Effect on Fish Species Diversity, AFATL-TR-70-81 (Eglin Air Force Base, Florida: Air Force Armament Laboratory, 1970).

21. M. Lloyd, J. H. Zar, and J. R. Karr, "On the Calculation of Information-Theoretical Measures of Diversity," The American Midland Naturalist, 79(2) (1968), pp 257-272.

22. R. R. Sokal and F. J. Rohlf, Biometry (San Francisco: W. H. Freeman and Company, 1969).

23. Kenneth D. Carlander, Handbook of Freshwater Fishery Biology (Ames, Iowa: Iowa State University Press, 1969), p 752.